A COMPARISON OF THE SKELETAL EFFECTS OF ESTROGENIC HORMONE IN VITAMIN C DEPLETED YOUNG AND OLD GUINEA PIGS

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TEN FIGURES

In young guinea pigs, estrogenic hormone inhibited skeletal growth; after several weeks, the growth processes were reactivated. In vitamin C-depleted young guinea pigs receiving estrogen, the onset of scorbutic bone lesions was delayed. However, skeletal scurvy developed when growth was resumed in the control animals treated with the hormone and fed the complete diet. Therefore, the arrest of growth was thought to be responsible for the retardation of the scorbutic sequence in vitamin C-depleted guinea pigs treated with estrogenic hormone (Silberberg and Silberberg, '48).

In an extension of these investigations, we have studied now the skeletal effects of estrogen in vitamin C-depleted older guinea pigs, in which epiphyseal growth and remodeling of the metaphysis proceed at a diminished rate or are at a standstill.

MATERIAL AND METHODS

The experiments were carried out in 30 guinea pigs of two different age groups. Group A consisted of 15 animals, 6 months old and weighing 450–500 gm; group B was com-

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posed of 15 animals about 1½ years of age and weighing 800–850 gm. The experiments were arranged as follows:

Group A

First series. Two guinea pigs were kept on a stock diet of Purina Rabbit Chow and water; vitamin C was supplied daily in the form of fresh lettuce.

Second series. Three guinea pigs received the stock diet and were injected subcutaneously with 0.5 mg of alpha estradiol benzoate [2] dissolved in sesame oil three times weekly one each for 3, 4, or 5 weeks.

Third series. Five guinea pigs were kept on a vitamin C free diet receiving Rabbit Chow and water without greens; two animals were sacrificed after 4, one after 5, one after 6, and one after 7 weeks.

Fourth series. Five guinea pigs were kept on the vitamin C free diet and received subcutaneous injections of 0.5 mg of alpha estradiol benzoate dissolved in sesame oil three times weekly. The guinea pigs were sacrificed in the following order: one after three, two after 4, and two after 5 weeks.

Group B

Fifth series. Two guinea pigs were kept on the complete diet and served as normal controls.

Sixth series. Three guinea pigs receiving the complete diet were injected with 0.5 mg of alpha estradiol benzoate in sesame oil three times weekly, one each for three, 4, or 5 weeks.

Seventh series. Five guinea pigs were fed the vitamin C free diet and were sacrificed as follows: one after two, two after three, and two after 4 weeks.

Eighth series. Five guinea pigs kept on the vitamin C free diet were injected with 0.5 mg of alpha estradiol benzoate in sesame oil three times weekly. They were sacrificed in the

[2] We are indebted to Dr. Erwin Schwenk of the Schering Corporation for the generous supply of Progynon-B.

following order: one after three, two after 4, and two after 5 weeks.

The weights of the animals were taken once a week. At the autopsy, the tibiae, femurs, ribs and vertebrae were fixed in 10% formalin, decalcified in 5% nitric acid, and embedded in paraffin. The sections were stained with hematoxylin and eosin.

GROSS OBSERVATIONS

The mean weights of the animals in the various groups are presented in table 1.

Showing the weights of the animals during the period of observation

Age-group A

		AFTER						
	INITIAL	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	5 WEEKS	6 WEEKS	7 WEEKS
Normal	498	531	556	595	611	626	637	689
Estrogen-injected	488	497	511	523	532	545	556	562
Vitamin C-depleted	493	429	407	393	327	316	308	235
Vitamin C-depleted and estrogen-injected	491	445	436	434	357	255	...	...

Age-group B

		AFTER				
	INITIAL	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	5 WEEKS
Normal	819	826	829	839	832	841
Estrogen-injected	822	824	819	808	789	792
Vitamin C-depleted	811	676	618	547	455	...
Vitamin C-depleted and estrogen-injected	806	718	643	551	465	450

Group A: Guinea pigs, 6 months of age

During 7 weeks of observation, the normal controls gained more weight (498–689 gm) than the guinea pigs receiving estrogenic hormone (488–562 gm). The bones of the latter animals were firmer than those of the untreated controls. The vitamin C depleted animals suffered a considerable loss of weight (493–235 gm); still, one was kept alive for as long as 7 weeks. The bones of these guinea pigs were thin and fragile. Intestinal, periosteal and periarticular hemorrhages were observed after 4 or 5 weeks. In the animal kept for 7 weeks, a slight swelling of the chondro-osseous junction of the ribs was seen. The vitamin C-depleted guinea pigs treated with the hormone lost during the first three weeks less weight (491–434 gm) than the non-injected deficient animals (493–393 gm). Subsequently, a drastic loss of weight occurred together with rapid deterioration in the animals' general condition. No guinea pig of this group survived 5 weeks. At autopsy, slight periosteal hemorrhages were noted. The bones were, however, firmer and not so fragile as in the deficient animals receiving no hormone. The chondro-osseous junctions of the ribs were not remarkable.

Group B: Guinea pigs, 1½ years of age

The weights of the normal controls fluctuated between 819 and 841 gm, whereas the estrogen-treated animals showed an insignificant loss of weight (822–792 gm). The bones of the latter animals were denser than those of the normal controls. The vitamin C deficient animals suffered a steady and rapid loss of weight (811–455 gm). After 4 weeks, the general conditions of the animals were so poor that they had to be sacrificed. At the autopsy, there were slight periosteal swelling and hemorrhages, but the chondro-osseous junctions of the ribs appeared unchanged. The weight loss of the vitamin C-depleted guinea pigs treated with estrogenic hormone was slower but, in the end, similar (806–450 gm) to that occurring in the non-injected vitamin C-depleted animals.

None of these guinea pigs survived the end of the 5th week. At autopsy, hemorrhages and edema were noted in the muscles.

HISTOLOGICAL EXAMINATION

The following description is based on the findings at the upper end of the tibia. Unless otherwise mentioned, conditions at the chondro-osseous junction of the ribs were similar to those seen in the tubular bone.

Group A: Guinea pigs, 6 months of age, and weighing 450–500 gm at the beginning of observation

First series. Normal control animals. The epiphyseal disks were 265 μ wide, and the cartilage cell rows were regularly arranged. They were separated from each other by abundant ground substance forming here and there wedge-shaped plugs. In the single cartilage cell row, 7 to 9 columnar and two or three hypertrophic cells were counted. The metaphysis was vascular, and numerous osteoblasts were present in the subepiphyseal layer. The trabeculae contained small osteocytes and were covered by a layer of ovoid osteoblasts. The bone marrow was hemopoietic. In the shaft (fig. 1), the cells and matrix were present in their usual proportions. The vascular canals perforating the cortex were medium wide. The endosteum was represented by a thin layer of connective tissue and a layer of osteoblasts facing the surface of the bone. The periosteum consisted of a vascular moderately dense connective tissue with many osteoblasts and osteoclasts situated in lacunar grooves of the compacta.

Second series. Estrogen-treated animals. The early changes consisted of increased hyalinization and condensation of the epiphyseal cartilage and increased deposition of osseous matrix. After injections of a total of 2.5 mg of estrogenic hormone over a period of 5 weeks, the growth zones were heavily calcified and narrowed (120 μ). In the single cartilage cell row, 4 or 5 columnar and one or two small cells of hypertrophic type were found. The cartilaginous ground sub-

stance was markedly increased in amount and density, and cracked plugs of calcified or ossifying cartilage traversed the epiphyseal plate. The metaphyseal capillaries were collapsed and embedded in a fibrous tissue; fewer vessels than ordinarily advanced from the bone marrow to erode the calcified cartilage cells. Much more bone than ordinarily was present in the metaphysis. The trabeculae were thick, broad-based and showed extensive calcification and interlacing. They were covered by a continuous layer of elongated spindle cells that predominated over the ovoid osteoblasts. The bone marrow was not remarkable. The shaft (fig. 2) was thickened and contained much calcium; the bony matrix was increased in amount. The vascular channels were comparatively narrow; they were filled with capillaries and connective tissue with some osteoblasts. The endosteum showed a discontinuous layer of small osteoblasts and more centrally a layer of spindle cells. The outer surface of the cortex was smoother and had fewer grooves than that of the non-injected controls. The periosteum was very dense and poorly vascularized; it contained few osteoblasts and only an occasional osteoclast.

Third series. Vitamin C-depleted animals. After three weeks' deficiency, the epiphyseal plate was 315 μ wide. The cartilage cells and the cartilaginous ground substance were swollen. In the individual cell row, 8 to 10 large columnar and two or three hypertrophic cells were counted which is about normal. The columnar cells were vacuolated, and the calcification of the hypertrophic cells was decreased. In the subepiphyseal layer, multinucleated giant cells and large osteoblasts were seen, but little osseous matrix was being formed. The metaphyseal capillaries were engorged. The trabeculae were thin but present in their usual number. Between the spicules, a loose fibrillar tissue containing elongated spindle cells was noted. The cortex of the shaft was thinned out. The vascular channels were dilated and filled with spindle cells and dilated capillaries. The bone marrow was unchanged. The endosteum consisted of one layer of osteoblasts and of

several layers of fibrocytes. The periosteum was vascular, loosened and composed almost entirely of spindle cells, whereas both osteoblasts and osteoclasts were less numerous than usual.

After 4 or 5 weeks deficiency (fig. 5), the growth zones were narrowed to 225 μ. The cartilage cell rows showed a disorderly arrangement. The cells were still more vacuolated than before, and the hypertrophic cells were barely recognizable as such. The cartilaginous ground substance was swollen and fibrillar. The metaphysis contained abundant loose connective tissue and dilated capillaries. Fully developed osteoblasts were absent. The trabeculae were markedly thinned out and shortened. Farther distally, they were fractured. Structural details became indistinct, and the bony fragments began to merge with the homogeneous eosinophilic (fibrinoid) material appearing in the metaphysis. The shaft (fig. 3) was porous and contained dilated vascular canals filled with loose fibrillar tissue. Both the endosteum and periosteum were edematous, and the latter was, in some places, detached from the surface of the bone; extravasation of blood was noticeable here and there.

With increasing duration of the experiment, the lesions in the subepiphyseal zone and the shaft spread and became more severe. After 6 or 7 weeks, the metaphysis showed large areas of breakdown of bone with marked fibrosis of the marrow and extensive fibrinoid change. The shaft was thinned out; the vascular pores were transformed into even larger spaces than in the earlier stages. Endosteal osteoblasts had disappeared, and extensive hemorrhages were now present in the edematous periosteum. The chondro-osseous junction of the ribs showed bead-like swelling owing to breakdown of the cortex and attempted callus formation. However, there was little if any new bone found. Instead, a loose fibrocytic tissue containing foci of hemorrhage had been formed.

Fourth series. Vitamin C-depleted animals receiving estrogenic hormone. In guinea pigs kept on the vitamin C-free diet for three weeks and given 1.5 mg of estrogenic hormone

during this period, the zones of endochondral ossification were 145 μ wide. The degree of calcification and hyalinization of the cartilage was comparable to that of the normal controls. In the single cartilage cell row, there were 5 to 7 columnar and two or three small cells of hypertrophic type. A good deal of bone was laid down underneath the cartilage. At their proximal ends, the spicules were thick and showed some interlacing. They were, however, thinner than in the normal controls; on the other hand, they were longer and thicker than in the vitamin C-depleted animals receiving no hormone. They were covered by a continuous layer of medium sized osteoblasts. The metaphysis was less vascularized than usual, and the perivascular connective tissue was cellular. No changes were observed in the bone marrow. The compacta of the shaft was densely calcified and contained small osteocytes. There were numerous and wide perforating vascular channels. The endosteum was not remarkable. The outer surface of the shaft was smooth; osteoblasts and lacunar grooves with osteoclasts were scanty. The periosteum was dense and contained many spindle cells.

After 5 weeks, when a total of 2.5 mg of estradiol benzoate had been administered, the growth zones measured 139 μ in width (fig. 6). The cartilage cells were decreased in number and size, but their arrangement was regular. The matrix between the cartilage cell rows was abundant and dense, and conversion into pre-osseous substance occurred in the hypertrophic cartilage cell layer. In contradistinction to the findings in vitamin C-depleted guinea pigs receiving no hormone, the spicules were well organized, fairly thick and showed transverse linking. The bone cells were prominent, and a good deal of bony matrix was present. The surface of the trabeculae was smooth, and there was no breakdown of bone; here and there, the spicules were covered by small osteoblasts, but more commonly by spindle cells. There were no fibrosis in the metaphysis and fibrinoid change was absent. The bone marrow was not remarkable. the shaft (fig. 4) was thicker and less porous, and the periosteum much denser

than in the vitamin C-depleted guinea pigs receiving no hormone. However, the Haversian canals were larger and contained more connective tissue than in the normal controls. There were only few and small osteoblasts in the endosteum and periosteum. Periosteal hemorrhages were absent.

Group B: Guinea pigs, 1½ years of age, and weighing 800–850 gm at the beginning of observation

Fifth series. Normal control animals. The former growth zones could be identified as a narrow transverse lamella of inert calcified and ossified cartilage; wide gaps indicated epiphyseo-diaphyseal union. Here and there, particularly at the periphery of the metaphysis, a slender long spicule was seen. The bone marrow was hemopoietic. The endosteum (fig. 7) consisted of a thin layer of fibroblasts and osteoblasts. The vascular channels perforating the cortex were filled with loose connective tissue. The periosteum was fibrillar and contained some osteoblasts and osteoclasts, the latter being situated in lacunae.

Sixth series. Estrogen-treated animals. After injections of a total of 2 mg of estrogenic hormone over a period of 4 weeks, the remaining epiphyseal cartilage was not affected by the treatment. The scanty metaphyseal trabeculae were denser and coarser than ordinarily. The endosteum of the shaft (fig. 8) was represented by a proximal layer of small osteoblasts and a central layer of spindle cells. The cortex was thickened, and the Haversian canals were narrowed. The periosteal surface was smooth and showed only few and shallow grooves. Osteoclasts were rare. The periosteum was thin but dense.

Seventh series. Vitamin C-depleted animals. After 4 or 5 weeks deficiency, the remnants of the epiphyseal cartilage and the metaphysis were unchanged. The cortex of the shaft (fig. 9) was of about the usual density. The endosteum was represented by a discontinuous layer of spindle cells; osteoblasts were practically lacking. The periosteum was detached from

the cortex and separated from the latter by extravasations of blood. Lacunar resorption of the bone by osteoclasts was in progress, and bony fragments could be detected in the periosteal tissue.

Eighth series. Vitamin C-depleted animals receiving estrogenic hormone. Administration of 2 mg of estradiol benzoate over a period of 4 weeks had no effect on the remaining epiphyseal cartilage and the scanty persisting spicules. The endosteum of the shaft (fig. 10) was covered by a discontinuous layer of small spindle cells. The Haversian canals were narrow. The surface of the shaft was smooth and contained, here and there, an osteoclast in a shallow groove. The periosteum was closely attached to the outer surface of the cortex and composed of fairly dense fibrous tissue. In contradistinction to conditions in the vitamin C-depleted animals receiving no hormone, there were no periosteal hemorrhages. After 5 weeks of injections, the findings were similar to those seen after 4 weeks.

DISCUSSION

In adult guinea pigs, in which the growth period was nearing its end, estrogenic hormone intensified the hyalinization and calcification of the epiphyseal cartilage; it decreased the resorptive processes acting on cartilage and bone and promoted both endochondral ossification and periosteal bone formation. In still older guinea pigs, in which skeletal growth had ceased, resorption of bone was likewise decreased under the influence of estrogenic hormone. The metaphyseal connective tissue was increased in amount, particularly if epiphyseo-diaphyseal union was still incomplete. The cortex was thickened and contained more ground substance than usual. The periosteal connective tissue was denser than ordinarily. The changes in the old guinea pigs were thus similar in kind but less accentuated than those observed in young adult animals, and in both these age groups the changes were less pronounced than in young rapidly growing guinea pigs. Similar differences in the skeletal response to estrogenic

hormone were noted in mice of various ages (Silberberg and Silberberg, '41).

In vitamin C-depleted adult guinea pigs, whose bones were still growing, though at a diminished rate, the skeletal manifestations of scurvy did not set in before the 5th week of the experiment, and more severe changes were seen only during the 6th week. In young fast growing vitamin C-depleted guinea pigs, the scorbutic lesions could first be observed after two weeks and they were fully developed as early as after three weeks. The delayed course of scurvy in the older guinea pigs was indicated not only by the later appearance of the skeletal changes but also by the longer survival of these animals as compared with that of rapidly growing young guinea pigs. Both these occurrences may be correlated to the decreased growth rate of older animals. In the young guinea pig, the remodeling of the metaphysis takes place at a rapid rate. The fast growing bones are, therefore, less firm than the bones of older animals in which skeletal remodeling is slow. Under adverse conditions such as vitamin C deficiency, the bones of the younger animal will, therefore, break down more readily than those of the older ones. This point could be proved experimentally: If the bone growth of young animals was inhibited by the administration of estrogenic hormone, the onset of the scorbutic lesions in the metaphysis was likewise delayed. The time of survival of animals fed a deficient diet depends upon the rate at which the vitamin stores of the organism are being exhausted. Very young guinea pigs require more vitamin C because of their high rate of growth. They, therefore, exhaust their vitamin C stores more quickly than young adult animals whose rate of growth is diminished. The additional requirements of the adult animals for maintenance are probably comparatively small. Thus, the young adult animal develops the signs of deficiency later than the younger animals. Old vitamin C-depleted guinea pigs did not live longer than 4 weeks. The shorter survival time of these animals is, however, not correlated to growth processes. These guinea pigs do not utilize

ascorbic acid as well as younger ones and succumb to the deficiency sooner than young adult individuals (Glasunow, '37; Sherman, '41, '43; Eddy and Dalldorf, '44; Brody, '45). At the time of death, some edema of the connective tissue, periosteal hemorrhages and slight osteoporosis were the only skeletal manifestations of scurvy. Possibly, the deficiency became incompatible with life before more severe skeletal lesions had a chance to develop.

In the skeleton of vitamin C-depleted young adult guinea pigs, the effect of injected estrogenic hormone was clearly noticeable: More bone was present in the metaphysis and in the shaft; there was no fibrinoid in the subepiphyseal layer, and edema of the connective tissue as well as periosteal hemorrhages were but slight. However, the time of survival of these animals was not lengthened by the administration of estrogenic hormone. It seems thus unlikely that the ascorbic acid metabolism itself might have been altered by the action of the hormone. The modification of the scorbutic sequence might rather be attributable to local effects exerted by the hormone. Again, the inhibition of both growth and resorptive processes following administration of estrogen may at least partly account for these findings. The growth-depressing action of the hormone might be of some significance, although the animals in this age group were nearing the end of their growth period. More important in the delay of the scorbutic lesions, however, would be the inhibition of bone resorption. Since no new bone is laid down in the absence of ascorbic acid, in the deficient animal the balance between bone formation and bone resorption is shifted in favor of the latter. Any factor inhibiting the resorption of bone such as estrogenic hormone will thus restore a more normal balance between bone formation and bone resorption. Local conditions may likewise be responsible for the antagonistic effects of ascorbic acid deficiency and estrogenic hormone in the connective tissue. The hyalinization of the latter caused by estrogen might at least temporarily prevent or delay the development of edema and the appearance of hemorrhages.

As discussed above, both estrogenic hormone and ascorbic acid deficiency affect the skeleton of old guinea pigs only to a slight degree. It is, therefore, not surprising that the combined treatment did not lead to conspicuous changes in the bones. Again in these old animals, the effect of the hormone manifested itself chiefly in the decrease of periosteal edema and hemorrhages and a slight increase in the density of the bone. As in the younger age group, these findings may be attributable to the hyalinization of the connective tissue and the decrease of resorptive processes both caused by the estrogen.

SUMMARY

As in rapidly growing young guinea pigs, administration of estrogenic hormone to vitamin C-depleted adult and old guinea pigs counteracts the skeletal effects of vitamin C deficiency. In animals of both age groups, this effect is primarily due to the inhibition of bone resorption. In the young adult guinea pig, suppression of growth caused by the hormone acts as an additional factor in the delay of the scorbutic sequence. The condensation of the connective tissue occurring under the influence of estrogen inhibits the appearance of edema and hemorrhages in the vitamin C-depleted animals of both age groups. The survival of these animals is not affected by the hormonal treatment suggesting that the antagonistic action of vitamin C deficiency and estrogen in the skeletal tissues is due to local factors and not to a modification of the ascorbic acid metabolism by the hormone.

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PLATE 1

EXPLANATION OF FIGURES

Section through the cortices of the tibia of male guinea pigs, 6 months of age at the beginning of observation.

1 Normal control. The cortex is well vascularized; the periosteum is thick and vascular; the endosteum consists of a layer of osteoblasts and a more central layer of fibroblasts. 88 ×.

2 Guinea pig injected with a total of 2.5 mg of alpha estradiol benzoate over a period of 5 weeks. The cortex is thicker than in the normal control (fig. 1). The Haversian canals are numerous but narrow; only a small layer of dense periosteum is present. The endosteum is composed of small osteoblasts and spindle cells. 88 ×.

3 Guinea pig kept on the vitamin C free diet for 5 weeks. The bone is markedly porotic, and the periosteum pronouncedly edematous. 88 ×.

4 Guinea pig kept on the vitamin C free diet and injected with a total of 2.5 mg of alpha estradiol benzoate over a period of 5 weeks. The thickness and vascularization of the shaft is intermediate between that in vitamin C-depleted (fig. 3) and estrogen-treated animals (fig. 2), resulting in an almost normal appearance. The periosteum is likewise of more normal structure.

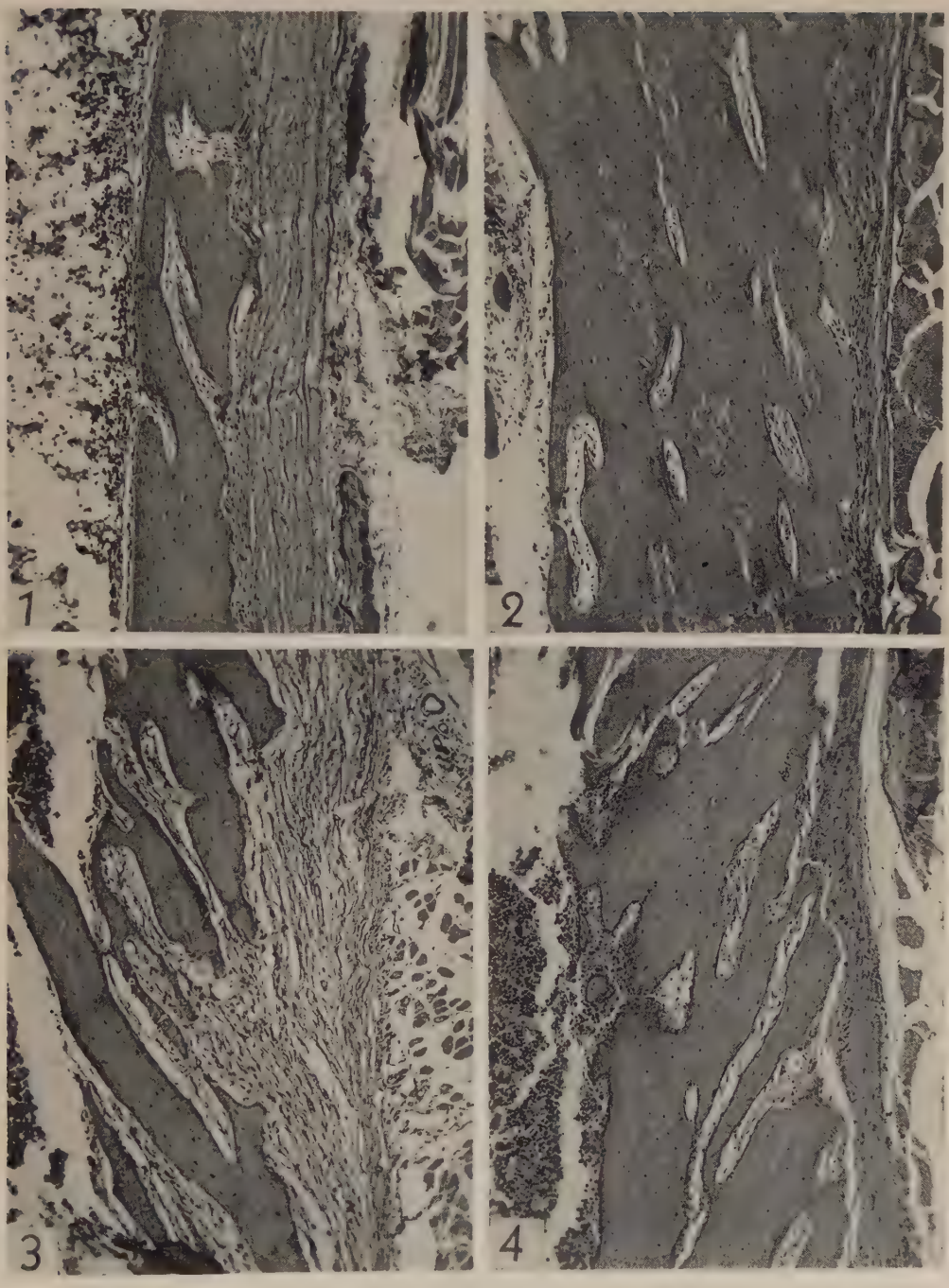
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PLATE 2

EXPLANATION OF FIGURES

Section through the growth zones at the upper end of the tibia of male guinea pigs 6 months of age at the beginning of observation.

5 Guinea pig kept on the vitamin C free diet for 5 weeks. The cartilage cell rows are arranged in a disorderly fashion; the columnar cells are vacuolated and the hypertrophic cells poorly developed. The primary spicules are short, thin and fragmented. The proximal metaphysis contains loose connective tissue, the distal metaphysis abundant fibrinoid material. 350 ×.

6 Guinea pig kept on the vitamin C free diet and injected with a total of 2.5 mg of alpha estradiol benzoate over a period of 5 weeks. The growth zone is narrower, the cartilage cell rows are more regularly arranged, and the cartilage cells and matrix are more compact than in the deficient animal receiving no hormone (fig. 5). The primary spicules are properly formed and contain a good deal of bony matrix. The amount of fibrous tissue in the metaphysis is about normal, and fibrinoid material is absent. 350 ×.

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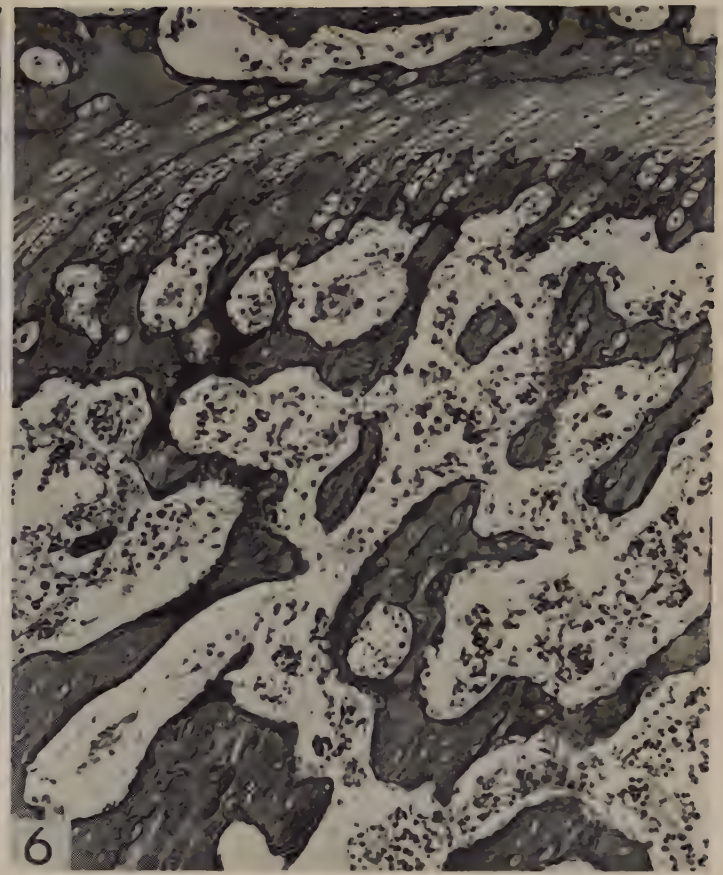
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PLATE 3

EXPLANATION OF FIGURES

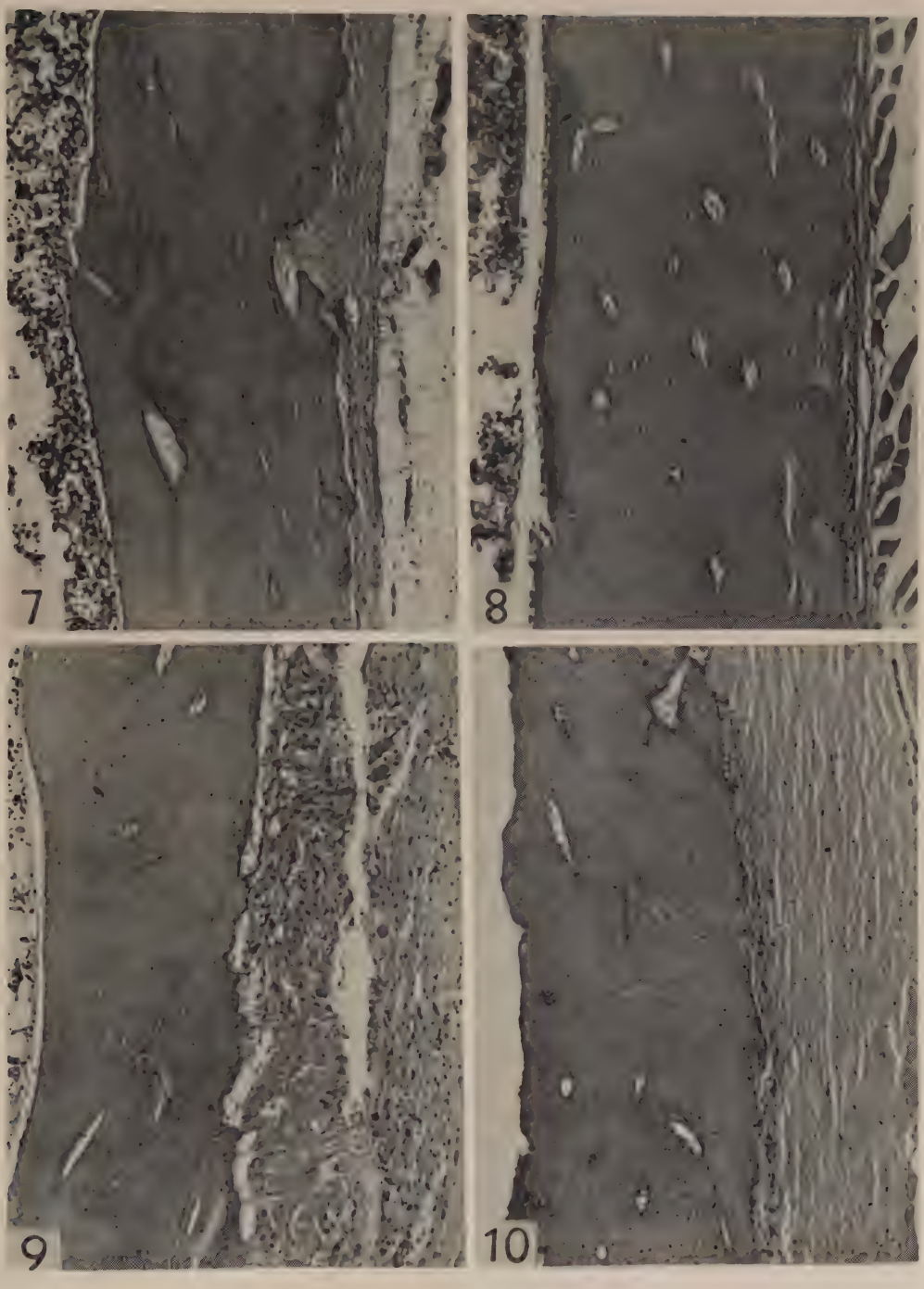
Section through the cortices of the tibia of male guinea pigs, 1½ years of age at the beginning of observation.

7 Normal control. The cortex is solid; the periosteal surface shows lacunar grooves, the periosteum is moderatly vascular; the endosteum contains some osteoblasts. 88 ×.

8 Guinea pig injected with a total of 2 mg of alpha estradiol benzoate over a period of 4 weeks. The cortex is slightly thicker and considerably smoother, but has narrower Haversian canals than ordinarily (fig. 7); the periosteum is thinner but denser than usual (fig. 7); the endosteum is composed of small osteoblasts and spindle cells. 88 ×.

9 Guinea pig kept on the vitamin C free diet for 4 weeks. The bone is of almost normal structure (fig. 7), but the periosteum is detached from the surface of the cortex and contains hemorrhages. 88 ×.

10 Guinea pig kept on the vitamin C free diet and injected with a total of 2 mg of alpha estradiol benzoate over a period of 4 weeks. The cortex appears normal. The periosteum is much denser than in the deficient animal receiving no hormone (fig. 9); it is closely attached to the surface of the bone and shows no hemorrhages. 88 ×.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THE INNERVATION OF THE ELBOW JOINT[1]

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TWO FIGURES

INTRODUCTION

The brief descriptions in current textbooks of anatomy of the nerve supply of the elbow joint appear to be based upon studies of gross distribution of articular nerves made during the last century. They provide no information relative to the intra-articular distribution of these nerves.

Cruveilhier (1844) mentioned that one of the branches of the musculocutaneous nerve perforates the biceps brachii muscle and passes to the elbow joint, but he said nothing of an articular branch from the nerve to the brachialis muscle. He described articular branches from other nerves, as follows: Several filaments from the median nerve loop around the termination of the brachial artery and enter the elbow joint. The ulnar nerve, as it passes between the medial condyle and the olecranon process gives a number of small twigs to the joint. The branch of the radial nerve to the long head of the triceps muscle can be traced to the olecranon process.

According to Rüdinger (1857) the articular nerves on the flexor side of the elbow joint arise from the radial, median and musculocutaneous nerves, and on the extensor side from the branch of the radial nerve to the lateral head of the triceps muscle and often from the branch to the medial head. The distribution of the articular nerves he described as fol-

[1] Aided by a grant from the Division of Research Grants and Fellowships, National Institute of Health, U. S. Public Health Service.

lows: Several branches from the median nerve enter the anterior region of the capsule, and an inconstant branch from the volar interosseus nerve runs recurrently between the radius and ulna and supplies the capsule around the head of the radius. The anterior region of the capsule is also reached by a branch of the radial nerve arising near the brachioradialis muscle. A branch or branches from the musculocutaneous nerve descend on or in the brachialis muscle and gain the anterior aspect of the capsule. The ulnar nerve has several articular branches. One arises some distance above the joint and descends to the posteromedial region of the capsule; a second branch arises behind the condyle and reaches approximately the same region as the first. A third and somewhat more distal branch supplies the middle part of the posterior region of the capsule and also gives fibers to the anconeus muscle. Nerves to the posterior region of the capsule about the olecranon process are also derived from the radial nerve, especially from its branches to the lateral and medial heads of the triceps muscle.

Sappey (1877) stated that the median nerve supplies one or more filaments to the medial region of the joint, and that the ulnar nerve gives an indeterminate number of extremely fine twigs to the joint, but he did not mention any articular branches of the radial or musculocutaneous nerves. Fick ('04) stated that the anterior region of the capsule is supplied by the radial, median and musculocutaneous nerves, and the posterior region by the radial (branch to the anconeus muscle) and ulnar nerves. The account in Quain's Elements of Anatomy ('09) appears to be based mainly upon the descriptions by Cruveilhier and by Rüdinger.

Piersol ('30) mentions an articular branch from the musculocutaneous nerve, and states that one or two twigs arise from the median nerve, as well as one or two from the ulnar nerve and several from the radial nerve. The accounts in Morris ('42), Gray ('42) and Cunningham ('43) differ very little from that of Piersol and are as brief.

No definitive studies were found in the literature since the time of Rüdinger (1857). Russian workers have made comprehensive studies of the nerve supply of the knee joint (Jeletsky, '31), hip joint (Sadovsky, '33; Geselevich, '35) and shoulder joint (Arkhangelsky, '31), but so far as could be determined they have not published any studies of the nerve supply of the elbow joint.

Ghetie ('39) describes branches from the ulnar and median nerves to the elbow joint of the horse, and Sasaoka ('38–'39) mentions a twig from the musculocutaneous nerve to the elbow joint of the cat. Aside from these brief accounts, there seem to be no references to studies in other animals.

MATERIALS AND METHODS

Nerves were dissected in 7 adult elbow joints so as to establish the general pattern of distribution. Five fetal joints were serially sectioned at 10 μ and stained with either hematoxylin and eosin or by a modified Masson's method.[2] One from a 10½-week fetus was sectioned in a transverse plane. One from an 8½-week fetus, two from a 12-week fetus and one from a 23-week fetus were sectioned in the sagittal plane. These ages are only approximate since they were determined on the basis of crown-rump measurements of fixed specimens. The sections included about half of the arm and forearm so that there was no difficulty in identifying peripheral nerves. While the material was not satisfactory for the study of nerve endings, even very small bundles of nerve fibers were easily traced so that it was possible to determine the intra-articular distribution of nerves.

Diagrammatic sketches were made of representative dissections and projection tracings were made of fetal sections which showed characteristic features of the nerve supply.

[2] The first 6 dissections were done while the author was in the Department of Anatomy, Stanford University, California, 1940–1941. The fetal material was obtained through the courtesy of Prof. C. H. Danforth, Department of Anatomy, Stanford University, California.

OBSERVATIONS

The elbow joint is supplied by the musculocutaneous, median, ulnar and radial nerves. The pattern of distribution is similar to that of the knee joint; that is, some of the articular nerves descend for considerable distances in the muscles of the arm while others arise in the immediate neighborhood of the joint.

In the illustrations, both of gross and microscopic material, branches are named according to their most specific origin. Thus, "median" (fig. 2b) indicates the articular twig from the median nerve.

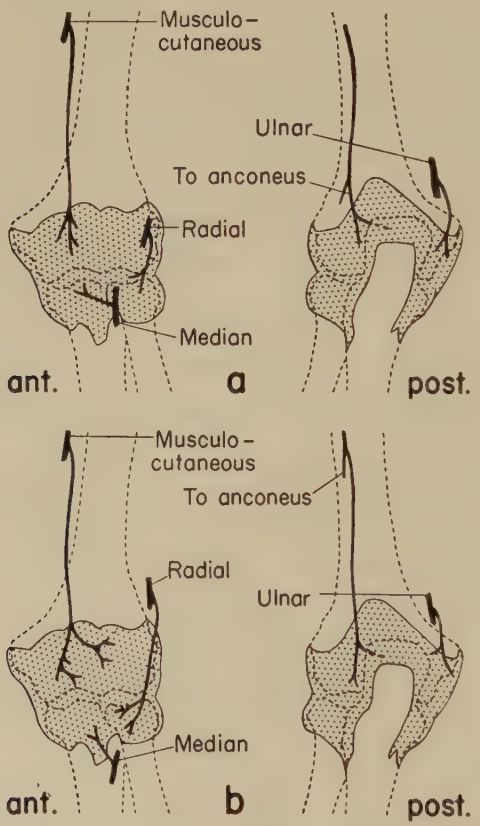

Fig. 1, a-b Anterior and posterior views of different types of distribution of nerves to the elbow joint. The articular capsule is represented by stippling.

The articular branches of the musculocutaneous nerve

In the middle third of the arm a small branch arises from the nerve to the brachialis muscle. This twig first descends on the medial edge of the muscle, but gradually turns deeply to gain the anterior aspect of the humerus, the periosteum of which it supplies. It reaches the elbow joint and divides into a variable number of branches which enter the anterior region of the joint capsule (fig. 1, ant.). In one instance this twig anastomosed with a branch from the median nerve and the combined trunk then entered the joint capsule (fig. 1d, ant.).

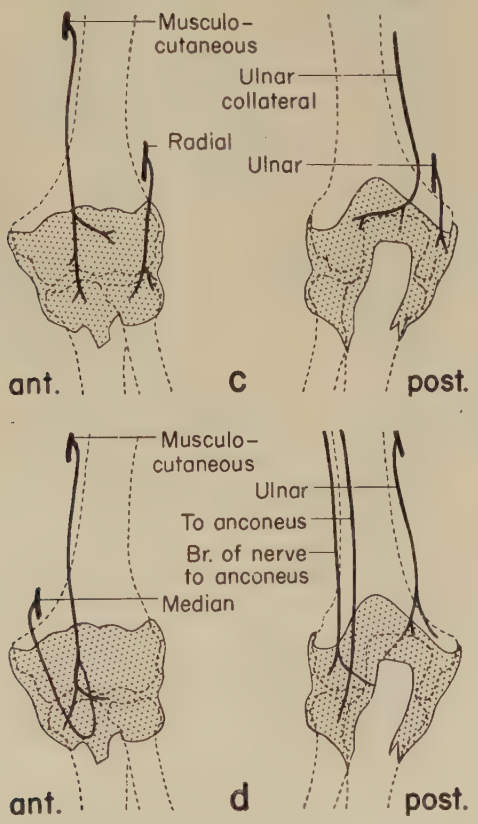

Fig. 1, c-d Further patterns of gross distribution.

A similar arrangement was found in the fetal joints. The articular branch descends along the medial edge of the muscle and usually penetrates the distal part of it to gain the capsule (fig. 2a-1). During its course it gives twigs to the periosteum

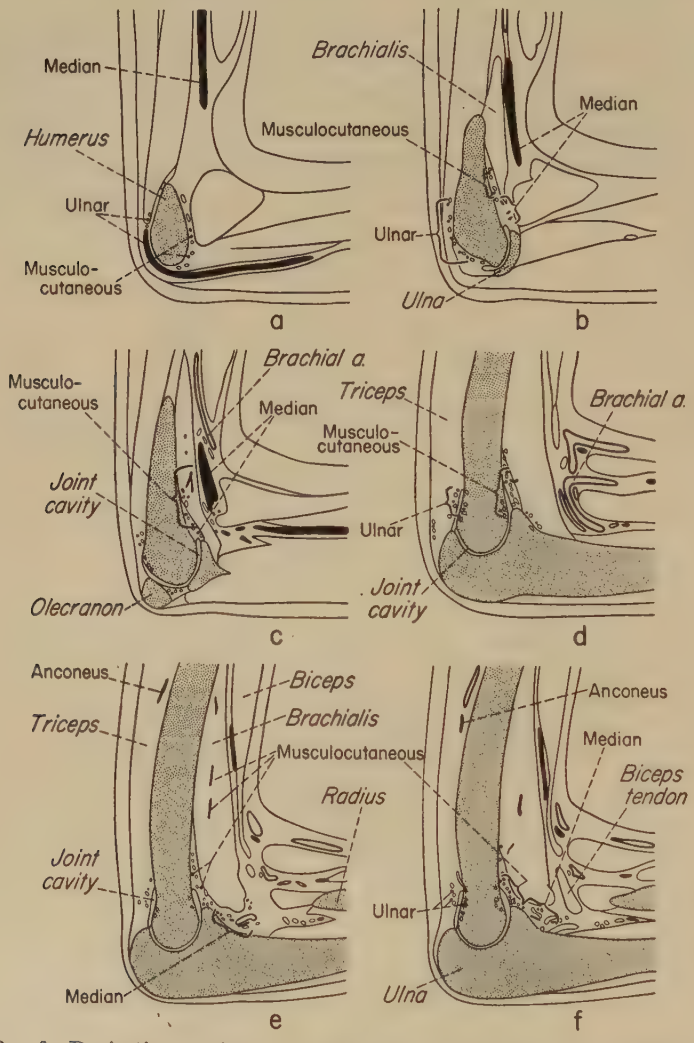

Fig. 2, a-f Projection tracings of a fetal joint (sectioned in the sagittal plane). Nerves are shown in solid black. The sections go from medial to lateral parts of the joint.

of the anterior aspect of the humerus. These twigs are closely associated with the blood vessels supplying the periosteum. In the distal part of the arm, the articular branch divides into a number of twigs which enter the anterior region of the capsule. These twigs spread medially and laterally in the fibrous layer of the capsule. Some of them appear to end in the connective tissue while others, in company with small blood vessels, penetrate more deeply into the synovial tissue. Some

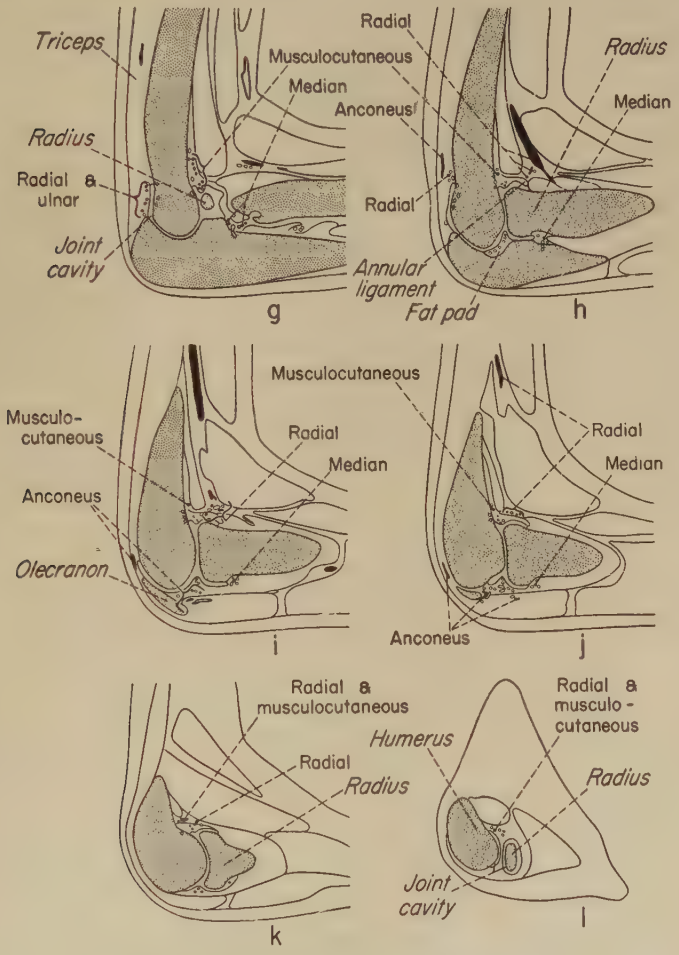

Fig. 2, g-l Continuation of the fetal sections.

of those twigs which course medially anastomose with small bundles of fibers from the median nerve, while of those coursing laterally, at least one usually anastomoses with an articular branch of the radial nerve (fig. 2h-1).

The articular branches of the median nerve

In one dissection no branches from the median nerve to the elbow joint were found. In the others, the level of origin of the articular branch was variable, the usual arrangement being as follows: Just before passing between the heads of the pronator teres muscle the median nerve gives a small twig which follows a slightly recurrent course to the capsule in the region of the medial epicondyle (fig. 1a-b, ant.). In one instance, the articular branch arose several centimeters proximal to the elbow joint and descended just distal to the bifurcation of the brachial artery. It passed posteriorly, between the ulnar and radial arteries, and then turned proximally and anastomosed with the articular branch from the musculocutaneous nerve (fig. 1d, ant.). The common trunk thus formed ramified in the anterior region of the capsule, somewhat to the medial side of the midline.

An articular branch of the volar interosseous nerve was not found in any of the dissections, although it was present in all the fetal joints.

In the fetal material, a small branch of the median nerve traverses the medial edge of the distal part of the brachialis muscle and enters the anterior region of the capsule (fig. 2b-c). This branch, or one of the twigs into which it divides, anastomoses with a twig from the articular branch of the musculocutaneous nerve and thus has a similar distribution. Another articular twig arises from the volar interosseous branch of the median nerve in the region of bifurcation of the brachial artery. It is closely associated with blood vessels as it follows a somewhat recurrent course between the radius and ulna (fig. 2c-j). Most of the fibers ramify in the periosteum of the coronoid process, but a few extend proximally to the lower attachment of the posterior region of the capsule.

They enter this portion of the capsule and reach almost to the fat pad along the lateral edge of the olecranon process (fig. 2g-j). This twig seems to correspond to the articular branch of the volar interosseous nerve which Rüdinger (1857) demonstrated by dissection.

The articular branches of the ulnar nerve

In one dissection no articular branches of the ulnar nerve were found. In the others, fine filaments arose from the ulnar nerve as it passed behind the medial epicondyle (fig. 1, post.). These entered the posteromedial region of the capsule and the ulnar collateral ligament. In one case the articular branch arose several centimeters proximal to the elbow joint and descended to the level of the upper part of the olecranon process. Here it turned laterally and entered the tissue composing the proximal extension of the posterior part of the capsule (fig. 1d, post.).

In the fetal joints the arrangement was as follows: As the ulnar nerve passes behind the medial epicondyle, one or more filaments supply the ulnar collateral ligament. Others extend laterally, in company with blood vessels, and ramify in the tissue forming the superior extension of the joint capsule (fig. 2a-g), supplying this as well as the adjacent portions of the humerus and olecranon process. The terminal portions of the small bundles of fibers reach as far as the area of distribution of the radial nerve.

The articular branches of the radial nerve

The distribution of this nerve to the elbow joint is extensive. While in its radial groove it usually gives a branch which descends in the lateral head of the triceps muscle, close to the humerus. Just proximal to the olecranon process it gives a fine branch to the capsule lining the olecranon fossa (fig. 1a, post.), while the main trunk continues to the anconeus muscle. In one case, a second branch of this nerve to the anconeus

arose in the arm and descended through the triceps to the posterolateral region of the capsule, somewhat proximal to the other articular branch (fig. 1d, post.). In two dissections, the ulnar collateral nerve, which arises from the radial nerve on the medial side of the arm, descended with the ulnar nerve and just proximal to the medial epicondyle turned sharply dorsalward through the substance of the triceps muscle. During this part of its course, several fine twigs were given to the capsule proximal to the olecranon process (fig. 1c, post.).

In all dissections except one, the radial nerve gave off a small twig after piercing the lateral intermuscular septum. In one case, this twig first supplied part of the brachialis muscle and then entered the radial collateral and the annular ligaments. In the other dissections in which it was present, it went directly to the anterolateral region of the capsule and to the anterior surface of the annular ligament.

In the fetal joints, it was observed that the articular twigs from the nerve to the anconeus accompany blood vessels into the posterolateral region of the capsule. Here they ramify in the tissue forming the anterior and posterior linings of the olecranon fossa and extend medially into the area of distribution of the ulnar nerve (fig. 2g-h). Some of the fibers accompany blood vessels into the adjacent portions of the humerus and olecranon process. The main trunk, on reaching the anconeus muscle, gives a twig which pierces the capsule and enters the vascular fat pad on the lateral side of the olecranon process (fig. 2i-j). This region may also be reached by fibers from the median nerve (see above).

As the radial nerve passes anterior to the elbow joint, it gives a branch to the anterior region of the capsule. This branch ramifies on the surface of the annular ligament (fig. 2h-l) and small bundles of fibers occasionally penetrate it. One small twig from this articular branch usually anastomoses with a laterally extending bundle of fibers from the musculocutaneous nerve.

DISCUSSION

The fundamental features of articular nerve supply are present in the elbow joint as they are in the knee, hip and shoulder joints (Gardner, '48). Each nerve supplies a definite region of the joint even though it varies in the manner in which it reaches the joint. There is a certain amount of overlap of articular nerves and there are regional differences in intra-articular distribution.

The articular branch of the musculocutaneous nerve is the most constant of the nerves supplying the elbow joint, both in gross and microscopic distribution. It supplies the anterior region of the capsule, and its area of distribution is overlapped by the median and radial nerves. The remaining nerves show much more variation in gross distribution, but nevertheless supply definite regions of the joint. Thus, the radial and ulnar nerves supply the posterolateral and posteromedial regions of the capsule, respectively, and overlap in their areas of distribution. In addition, the radial nerve supplies the fat pad along the lateral edge of the olecranon process and there is a distinct possibility that fibers from the median nerve also extend into this area. It is difficult to say how constantly the latter fibers are present. They were found in all the fetal joints and undoubtedly occur in the adult since Rüdinger found them in some of his dissections. Other articular branches of the median and radial nerves supplied the medial and lateral portions of the anterior region of the capsule and overlapped the area supplied by the musculocutaneous nerve. It is apparent, therefore, that each major region of the joint is supplied by more than one nerve.

The elbow joint is similar to the knee joint in the manner in which various nerves reach it. The olecranon process is homologous to the patella, and proximal to it is an extension of the joint cavity just as there is a suprapatellar extension of the knee joint cavity. The fat pad inferior and lateral to the olecranon process resembles the infrapatellar fat pad, not only in adipose tissue, but also in the vascular network it contains. Nerves descending in the arm reach the olecranon

fossa, and also bypass the olecranon process to reach the fat pad, just as nerves bypass the patella to enter the infrapatellar pad.

In the knee joint, the greatest concentration of nerve fibers occurs in the posterior region of the capsule. A similar concentration is present in the anterior region of the capsule of the elbow joint. In both cases, these regions of the capsule are the ones most subject to compression or change of some sort during movement. In these same regions, many of the bundles of fibers ramify mainly in the fibrous layer of the capsule. Other bundles of fibers here, but more especially in other regions, such as the fat pad, are closely associated with the blood vessels supplying the capsule and neighboring bone.

The functional implications of articular nerve supply were discussed in more detail in the study of the nerve supply of the knee joint (Gardner, '48).

SUMMARY

The nerve supply of the human elbow joint was studied in dissections of adult joints and in serial sections of fetal joints. The articular nerves are derived from the musculocutaneous, median, ulnar and radial nerves.

The musculocutaneous nerve supplies the periosteum of the humerus and the anterior region of the capsule. Twigs from its articular branch anastomose with others from the radial and median nerves.

The median nerve supplies the anteromedial region of the capsule and overlaps the area supplied by the musculocutaneous nerve. A small filament from the volar interosseous nerve supplies the posteroinferior region of the capsule and the adjacent portion of the ulna and extends proximally, almost as far as the fat pad along the lateral edge of the olecranon process.

The ulnar nerve supplies the posteromedial region of the capsule and the neighboring portions of the humerus and olecranon process. It overlaps the area of distribution of the radial nerve.

The radial nerve, by way of its branch to the anconeus and occasionally from other branches, such as the ulnar collateral nerve, supplies the posterolateral region of the capsule, the neighboring portions of the humerus and olecranon process and the fat pad; it overlaps the area supplied by the ulnar nerve and probably by the median nerve. Another of its articular branches ramifies in the surface of the annular ligament and anastomoses with a branch from the musculocutaneous nerve.

There were more nerve fibers in the anterior region of the capsule than elsewhere in the joint and many of these appeared to be confined to the fibrous layer of the capsule. The majority of nerve fibers supplying the joint were closely associated with the blood vessels supplying the capsule, epiphyses and fat pad.

ACKNOWLEDGMENTS

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THE AFFINITY OF SYNCYTIUM, FIBRIN AND FIBRINOID OF THE HUMAN PLACENTA FOR ACID AND BASIC DYES UNDER CONTROLLED CONDITIONS OF STAINING [1, 2]

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FOURTEEN FIGURES

Formed protein elements of cells and tissues may be characterized by measuring the degree of their affinity for acid and basic dyes. For this purpose serial histological sections are stained at successive pH, other conditions being maintained constant (Singer and Morrison, '46; Dempsey and Singer, '46; Dempsey et al., '47). By this method human placentas of various ages have been investigated with reference to the staining characteristics of the trophoblastic syncytium, fibrin and fibrinoid.

MATERIALS AND METHODS

Four human placentas, one of 5 weeks, two of 15 weeks and one of term, were investigated. Pieces of these placentas were fixed in Zenker's acetic solution, sectioned at 5 μ, mounted on slides and stained in most instances with either orange G (an acid dye) or methylene blue (a basic dye). In some cases, acid dyes other than orange G were used. Staining was carried out in large volumes of solution (1500 cm^3) so as to

[1] Reported at the meeting of the American Association of Anatomists (Singer and Wislocki, '47).

[2] This work was done in part under a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

avoid any significant decrease in concentration of the dye as staining proceeded. The pH of the solutions ranged in steps of approximately 0.5 from pH 1.5 to 8.0. The ionic strength of the dye solution was 0.01, the molarity of the dye concentration 5×10^{-4}, and the temperature of the solution, 25 ± 0.1°C. These conditions were selected on the basis of previous physico-chemical studies of protein-dye interactions (Singer and Morrison, '46; '48). In controlling the pH and ionic strength (Green, '33) a phosphate buffer was used between pH 8.0 and 5.5, an acetate system between pH 5.5 and 3.8 and hydrochloric acid with added sodium chloride below 3.8. Previous experiences (Singer and Morrison, '48) have shown that use of these systems, which is demanded by the wide pH range, introduces no important variable in the study. A succession of beakers containing the solutions was placed in a constant temperature bath, and the slides upon which the sections were mounted were placed in the beakers. Sections were stained progressively for 24 hours in order to insure equilibrium between stained tissue and dye solution; the solutions were mechanically stirred during this time. The sections were then dehydrated by brief immersion in solutions of graded concentration of tertiary butyl alcohol rather than ethyl alcohol so as to avoid excessive loss of dye (Levine, '39). Thus a succession of slides was obtained, stained at successive pH with either orange G or methylene blue.

In order to assess the degree of protein-dye interaction, the light absorption of a given structure was measured on successive slides stained at successive pH. The image of the object in question was projected with an ordinary compound microscope onto a ground glass focussing screen of a photomicrographic camera, and its staining intensity measured with a photometric scanner. Details of this method together with a photograph of the equipment have been presented in a previous paper (Dempsey and others, '47). As a source of light an ordinary bull's eye microscope lamp was employed and green and yellow filters used depending upon whether the

sections had been stained with orange G or methylene blue. The light transmitted through the slide and cover slip was adjusted to give a reading of 100 on the photometer. The slide was then moved until the desired structure appeared in the field whereupon the light transmission of the structure was measured with the photometric scanner. The per cent of light absorbed by the dye was taken as the difference between the initial and the final transmission [3] and the absorption due to dye uptake was then plotted against pH. Curves obtained in this manner served as an index of the dye-protein interaction. Ordinarily an objective of 44 × magnification and an ocular of 15 × magnification were used; however, individual zones of the trophoblastic syncytium had to be studied at a higher magnification (90 × oil immersion lens). Because of the limitations in light intensity at the higher magnification, filters could not be used. Comparison of curves obtained using filters at lower magnifications with those obtained without filters at higher magnification showed, as might be expected, that similar conclusions may be derived although at any given pH the per cent absorption was higher when filters were used.

PLACENTAL SYNCYTIUM

The cytoplasm clothing the placental villi can be stained with either acid or basic dyes depending on the pH at which staining is carried out. At high pH, the syncytial trophoblast covering the villi shows its greatest capacity to bind methylene blue and its least affinity for orange G. With diminishing pH there is a decrease in affinity for the basic dye and an increased uptake of the acid dye. These relations, observed in all placentas, are exemplified in figure 4 for the placenta at term and are generally characteristic of the interactions of dye and solid protein (Singer and Morrison, '48). The degree of staining follows the expected alteration in charge on proteins with variation in pH. At high pH values the net negative

[3] Actually, however, unstained tissue absorbs a small but definite amount of light. Since this error is constant for all readings and does not alter substantially our conclusions, no further reference will be made to it.

charge on the protein is high and the affinity for cationic (basic) dye is greatest. With decrease in net negative charge as the pH is lowered, basic dye-binding falls off rapidly until no staining occurs. On the other hand, at low pH values where the net positive charge on the protein is high, anionic (acid)

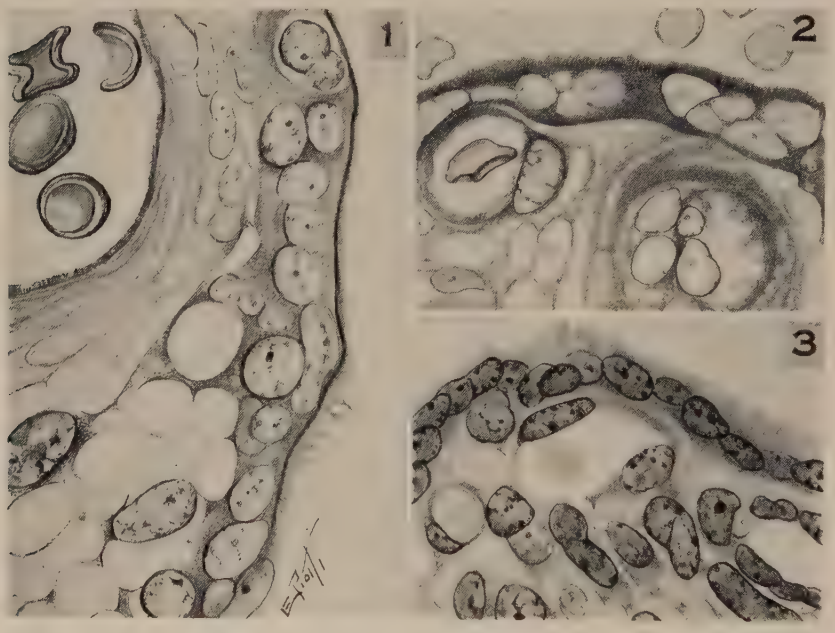

Fig. 1 Drawing of syncytium of 15 weeks showing three zones of staining with orange G (pH 4.4). Oil immersion objective and 10 × ocular used in drawing.

Fig. 2 Drawing of syncytium of term stained with orange G at pH 4.4. Except for suggestion of intermediate zone, syncytium stains relatively uniformly. Oil immersion objective and 10 × ocular.

Fig. 3 Drawing of syncytium of term stained with methylene blue at pH 7.3 showing relatively uniform basophilia and absence of zonation. Oil immersion objective and 10 × ocular.

dye is bound most strongly. As the pH is raised, binding of acid dye diminishes and this fall accompanies a progressive decrease in the net positive charge on the protein. There are pH levels, however, where either acid or basic dye may be bound (fig. 4), suggesting that some dye is bound even when

the net charge on the protein is of the same sign as that on the dye (Singer and Morrison, '48).

Although all parts of the trophoblastic syncytium interact with acid or basic dye, the degree of interaction at a given pH varies considerably from one locus to another. Another variable which influences notably the dye-binding capacity of the syncytium is the placental age. These factors are considered in the following sections for each of the dyes.

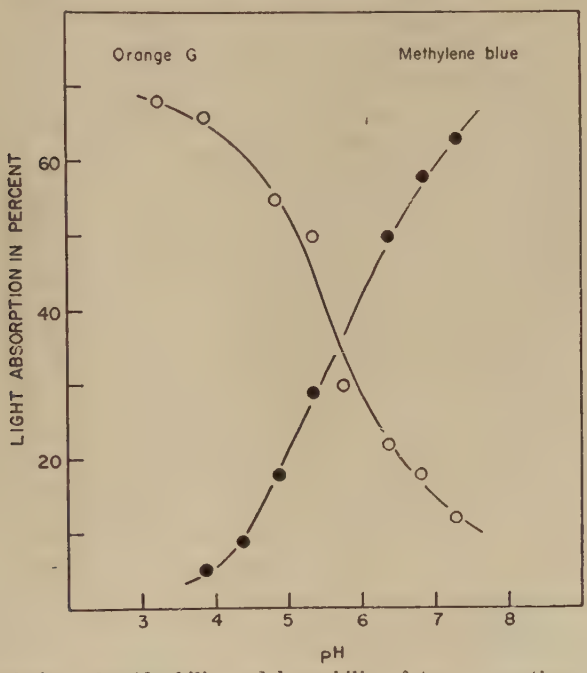

Fig. 4 Relation of acidophilia and basophilia of term syncytium to pH.

The interaction of syncytium and acid dye. The syncytium does not stain uniformly with acid dye, but shows various zones of staining. The syncytium of the placenta of 15 weeks shows the most pronounced zonation and, therefore, will be described first. In the placenta of 15 weeks three zones are evident in the syncytium stained with orange G at low pH (fig. 1, middle). There is an outermost zone which is weakly acidophilic and corresponds in position and structure to the

brush border. The cytoplasm of the innermost, nucleated region of the syncytium is somewhat more acidophilic. Between these two zones there is an intermediate zone which is in most instances sharply set off from the other two by its intense affinity for orange G. This differentiation of the syncytium into three distinct zones is evident over a wide range of pH (fig. 5). With increasing pH the acidophilia of each of these regions is suppressed (fig. 5) in the order of

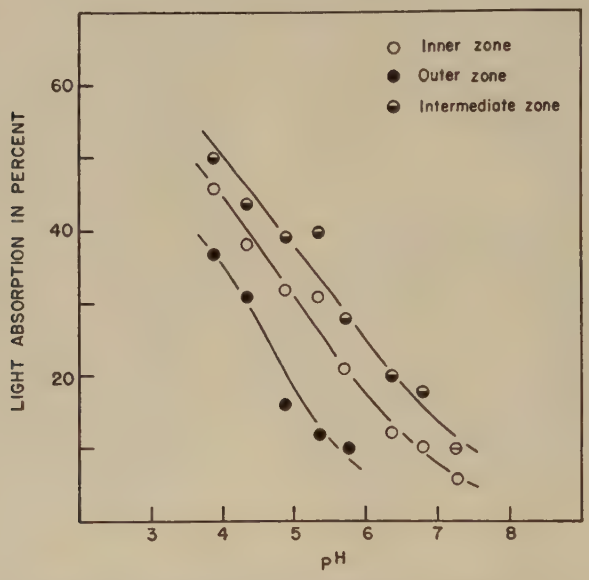

Fig. 5 The staining characteristics of the three acidophilia zones of the 15 week syncytium. Orange G.

their staining affinities, with the brush border disappearing first (pH 6.0) followed in turn by the inner zone (pH 7.5) and the intermediate one (pH 8.0). Tinctorial zonation is not observed in all villi nor always in all parts of the same villus. Since a brush border is not uniformly present (Wislocki and Bennett, '43), the region of high acidophilia may lie at the surface (fig. 1, upper part), but other places may lack the intermediate zone as well. In such spots the syncytium is relatively uniformly stained (fig. 1, lowermost part). And, finally,

in regions showing three zones there may be differences in the relative prominence of each zone. Similar zonation of the 15 week placenta was observed after using other acid dyes, for example, light green, azocarmine, aniline blue and phloxine B.

In the placenta of 5 weeks, zonation of the syncytium with respect to binding of acid dyes and the order of affinities of the three zones are comparable to conditions observed in the 15 week placenta. However, the innermost and the intermediate zones are less well differentiated, tending especially at high pH to stain as 1 (fig. 11). Indeed, each region of the syncytium of the 15 week placenta shows a greater capacity for binding acid dye than does the corresponding zone of the 5 week one. At any given pH the intensity of staining was greater for the older placenta, although staining was performed under precisely the same conditions. The increase in acidophilia of the syncytium with age is especially apparent when the pH of dye suppression is compared for the two placentas. There is no staining above pH 6 in the 5 week placenta, whereas suppression of staining capacity in the older one does not occur until a pH of 8 is attained.

In the placenta at term, zonation of the syncytium is not very obvious (fig. 12), although occasionally there is a suggestion of an intermediate zone and an outer border (fig. 2); but these are too inconstant and narrow to be followed photometrically. Consequently, the thin syncytial covering of the term placenta may be best compared with the inner cytoplasmic zone of the earlier placenta. The affinity of the term syncytium for acid dye surpasses that of the inner syncytial zone of the 15 week placenta, but it resembles fairly closely the staining reaction of the intermediate zone at 15 weeks. This resemblance is of interest since it suggests that the high affinity for acid dye which distinguishes the intermediate area reaches a maximum by about 15 weeks and then spreads into the innermost zone until by term the acid staining properties of these two areas become indistinguishable (compare intermediate zone of figure 5 with syncytium of figure 6).

The increase in the acidophilia of the syncytium with age is exemplified in figure 6 where the "signatures" of the inner zone of the early placenta and the thin syncytium of term are compared. There is a difference in pH of dye suppression of approximately 1.5 and the intensity of dye uptake at any given pH is greater for syncytium at term.

The change in binding of acid dye which occurs in the ageing placenta may be visualized by plotting the light absorption

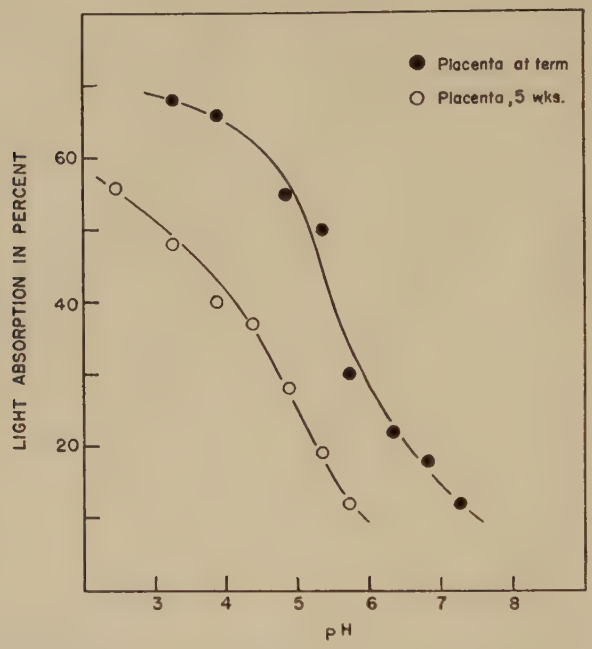

Fig. 6 Comparison of the acidophilia of the inner zone of the 5 week placenta and of the syncytium of term.

at a selected pH against the age for any of the syncytial zones. Such a plot is shown in figure 7 for the innermost zone at pH 5.0 and defines the rate of change of acidophilia with age. According to the curve of orange G uptake the change is most rapid in the early months of pregnancy. By the middle of pregnancy the major increase in acidophilia has occurred. The rate of change for the intermediate zone is probably more

rapid initially, although the intermediate and inner zones become relatively indistinguishable in later development.

The interaction of syncytium and basic dye. Differences are also apparent in the staining of the syncytium with methylene blue, but in contrast to the acidophilic reactions only two zones of staining are visualized. In the placentas of 5 to 15 weeks (figs. 8 and 13), there is an outer zone that stains lightly with the basic dye and a deeper area including the

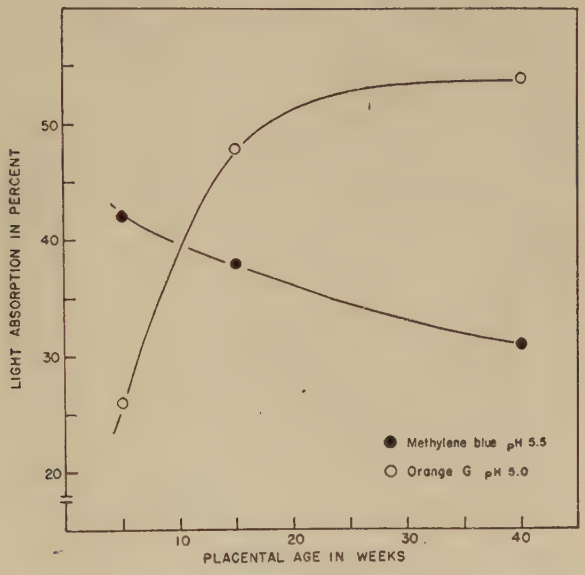

Fig. 7 Change in acidophilia and basophilia of the syncytium with age at a selected pH.

nuclear region which is much more basophilic. The inner zone corresponds in size as well as position to the innermost zone of syncytium visualized with orange G and is approximately three or 4 times the size of the outer zone. The outer zone would seem to include both the brush border and the intermediate zone as seen with acid stains, since it is wider than either of the latter zones. At term, although a thin outer margin of weak basophilia can often be observed, a distinct outer basophilic zone such as is seen in the younger

placentas is seldom found (figs. 3 and 14). In most places the syncytium at term stains quite uniformly and, therefore, a single curve (fig. 4) describes its basophilia.

Comparison of the staining of the syncytium at different ages reveals a continuous decline in basophilia. The curves of basophilia of the inner syncytial zone of the 5 week placenta and of the syncytium at term are plotted in figure 9 where it is observed that at any selected pH the uptake of basic dye

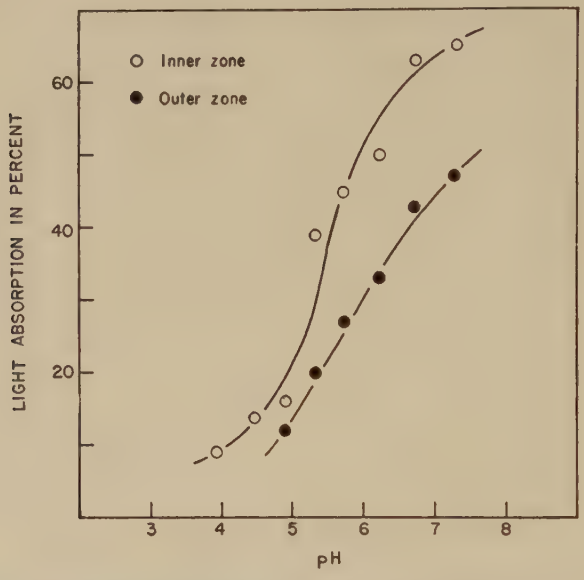

Fig. 8 The staining characteristics of the inner and outer zones of the syncytium as visualized with methylene blue.

is greater in the younger placenta. The decline in basophilia is especially apparent in figure 7 where the light absorption at a selected pH of 5.5 is plotted against the age for the inner zone of the syncytium.

The cytoplasmic basophilia of the nuclear zone of the syncytium appears to be referable to ribonucleoprotein. In figure 9 a curve of the staining of nucleoprotein of Nissl substance is compared with curves of the inner syncytial zone of the 5-week placenta and the thinned-out syncytium of the

term placenta. The capacity to bind basic dye sets in at pH 4.0 and rises sharply above this value. The greatest rate of change occurs over a similar pH range in the three curves. The similarities in the curve can be explained on the basis of similarities in the dissociating characteristics of the proteins. A major difference in the staining of Nissl substance and syncytial cytoplasm is that the curve of placental baso-

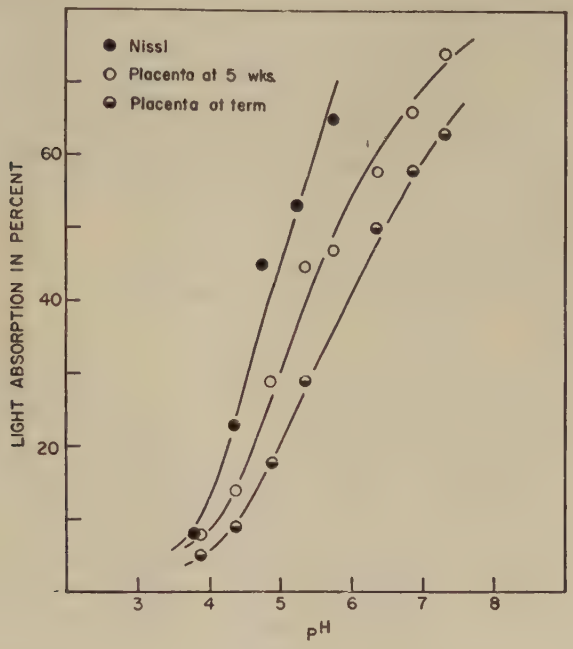

Fig. 9 Comparison of the basophilia of the inner syncytial areas of the early and late placenta and of the ribonucleoprotein of Nissl substance.

philia does not rise as rapidly as that of Nissl substance, suggesting that the concentration of ribonucleoprotein per unit volume of syncytial cytoplasm is less than that of Nissl substance. The identification of syncytial basophilia with that of ribonucleoprotein concurs with the conclusion reached by Dempsey and Wislocki ('45). They showed that the basophilia of syncytial cytoplasm as visualized in the eosin-methylene blue technique is abolished by previous exposure of the

sections to ribonuclease. They also reported a decline with age in the basophilia of the syncytial cytoplasm and attributed this to a gradual decrease in ribonucleic acid — a view supported by results described above.

FIBRIN AND FIBRINOID

Associated with the cellular trophoblast of the cell islands and cell columns of the placenta in the form of a ground sub-

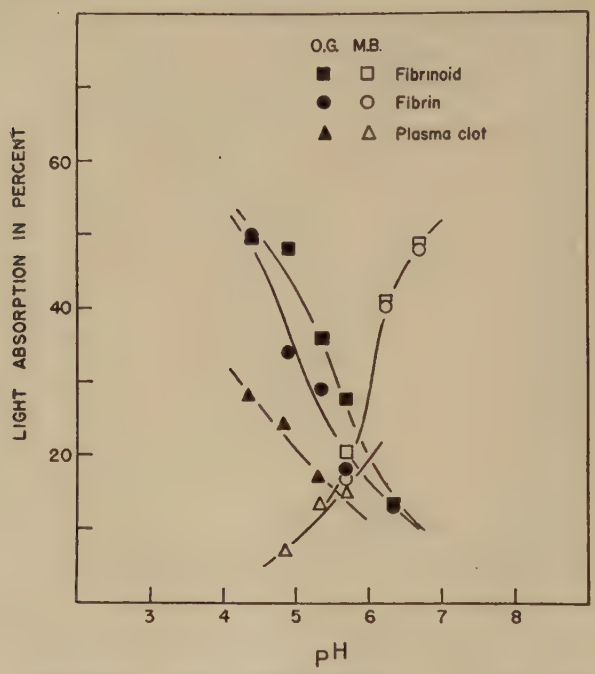

Fig. 10 A comparison of the staining capacity of placental fibrin, fibrinoid and a plasma clot. O. G., orange G; M. B., methylene blue.

stance are two noncellular materials known as fibrin and fibrinoid. These substances often occur together, one grading into the other. A salient morphological characteristic which distinguishes one from the other is that fibrin is fibrillar or granular, whereas fibrinoid is homogeneous and glassy in appearance. Fibrin and fibrinoid also show distinctive dye affinities when stained with triacid stains such as Mallory's or

Masson's. Placental fibrin stains with the red colloidal dye (acid fuchsin or azocarmine), whereas fibrinoid takes up other colored components of the staining solution (Wislocki and Bennett, '43). Opinions differ as to the nature of these substances as well as their genesis (see review of Wislocki

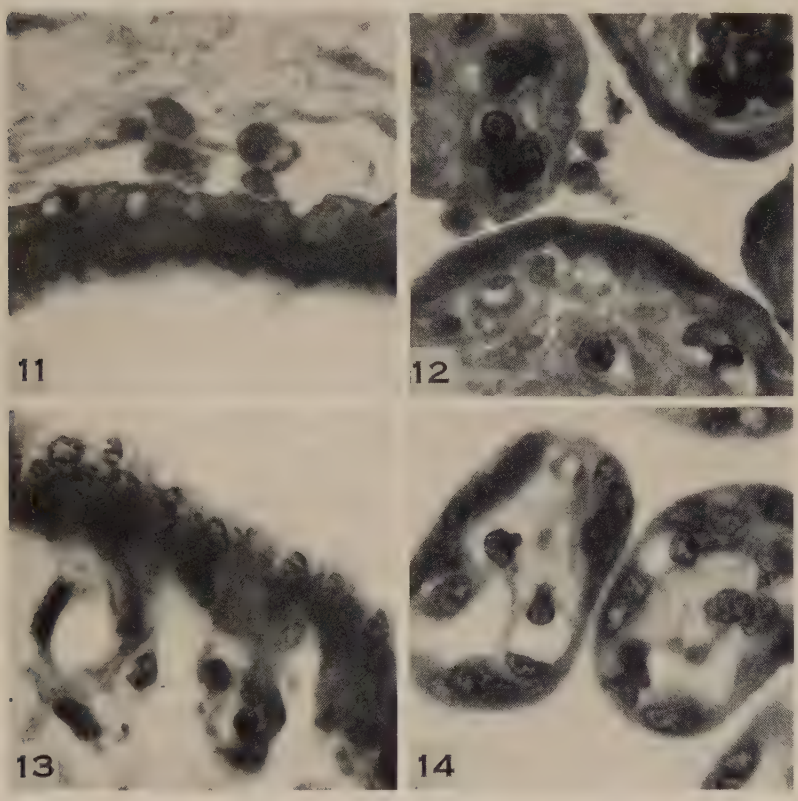

Fig. 11 Syncytium of 5 week placenta stained with orange G at pH 1.5. Note the weak acidophilia of the brush border and the suggestion of an intermediate highly acidophilic zone. × about 1600.

Fig. 12 Syncytium of term placenta showing relatively uniform staining and high acidophilia. Orange G, pH 1.5. × about 1600.

Fig. 13 Syncytium (15 weeks) stained with methylene blue at pH 7.3. The most basophilic region is that around the nuclei and the least is the outer area. × about 1600.

Fig. 14 Syncytium of term placenta. Methylene blue, pH 7.3. Note uniform staining of syncytium and relatively weak basophilia as compared to inner syncytium of figure 13. × about 1600.

and Bennett, '43). There is a general belief that fibrinoid arises as an autolysate of placental tissue although different views are held on the contributing sources. Wislocki and Bennett ('43) have expressed the belief that fibrinoid contains, in addition to products of placental tissue, various contributions from the maternal tissues and blood stream including a fibrin component.

In the hope that some light might be thrown on the chemical nature of these substances, the interaction of placental fibrin and fibrinoid with acid and basic dyes was observed. In selection of the areas to be read photometrically only those structures were chosen in which extremes of glossy (fibrinoid) or fibrillar (fibrin) appearance were evident. Finally, the staining characteristics of a clot of human plasma subjected to the same histological procedures as for placental tissue were compared with those of fibrin and fibrinoid. Because of the highly fibrillar character of the plasma clot and the fibrin of the placenta, allowing the passage of unabsorbed light through their interstices, the curves for these substances could only be compared over a restricted region, namely, where there was most rapid change in dye-uptake with variations in pH.

Results of these studies (fig. 10) show that the staining characteristics of fibrin and fibrinoid are very similar. In both instances basophilia appeared approximately at the same pH (4.5 to 5.0) and acidophilia was evident at pH 6.0 to 6.5 and below. The staining characteristics of fibrin and fibrinoid showed little variation from one region to another in the placenta. Moreover, although there is a progressive increase in the amount of fibrin relative to that of fibrinoid (Wislocki and Bennett, '43), no essential change in acidophilia and basophilia of these substances appears in placentas of different ages. The staining curves for a plasma clot show a general similarity to those of fibrin and fibrinoid (fig. 10); the pH of suppression of acid or basic staining deviates from values of the latter substances by a fraction of a pH unit.

However, the affinity at any prescribed pH is less than that of either fibrin or fibrinoid, but this difference may be attributed in large part to the highly reticular character of the clot.

DISCUSSION

Placental syncytium. Zonal differences had been previously observed in syncytium after staining with a triple acid mixture or with eosin and methylene blue (Wislocki and Bennett, '43; Dempsey and Wislocki, '45). The brush border and deeper syncytical regions are selectively stained in the triacid technique; after Masson's triple stain the outer border appears green whereas deeper parts are reddish and after Mallory's the colors are respectively blue and purplish. With eosin-methylene blue, the brush border is invariably red or pink and the underlying portions blue especially in younger placentas. However, no further differentiation of a third intermediate zone, such as reported here, has been observed with these stains.

The tinctorial zonation evidently depends on fundamental regional differences in the proteins of the syncytium. These differences may be various, but two possibilities appear especially likely. First, there might be zonal differences in concentration of stainable protein per unit volume of cytoplasm. When the concentration of the protein is high, the affinity of that region for acid and basic dyes would also be high, and, contrariwise, low dye-binding would imply a low concentration of protein. Second, there might be regional chemical differences in the constituent proteins so that each region of the syncytium would be differentially stained. Proteins having different dissociation characteristics would bind different quantities of dye under similar staining conditions. These two factors — regional differences in the concentration of protein and in the chemical nature of the protein — may operate in establishing the zonation of the syncytial cytoplasm. The low affinity of the brush border for acid and basic dyes may be mainly due to difference in the concentration of stainable protein. The morphology of the brush border

would support such a postulate since it is foamy and vacuolated and its general appearance suggests that fluid may be imbibed here or that secretory or excretory products as they leave are accumulated at this point (Wislocki and Bennett, '43). Such concentration of substances in vacuoles in the general mesh of the protoplasm would in effect lower the unit concentration of solid, stainable protein. In the case of the two deeper zones of the syncytium chemical differences are more evident than differences in concentration. Were the high acidophilia of the intermediate zone due to greater concentration of protein, the affinity for basic dye should be correspondingly high. On the contrary, an intermediate zone is not distinguishable with basic dye and the basophilia appears similar to that of the brush border. The high acidophilia and low basophilia of the intermediate zone suggest that the available net positive charge on the proteins of this locality is much greater than elsewhere in the syncytium and the net negative charge, responsible for basic dye affinity, is lower than for the inner zone. The staining of the inner perinuclear zone of the syncytium can be explained in part on its ribonucleic acid content. Ribonucleic acid exists in highest concentration here rendering the available net negative charge of this region of the syncytium greatest. The high acid dissociation of ribonucleoprotein accounts for the marked affinity of the perinuclear zone for basic dye.

The changes in dye affinity which occur with ageing of the placenta can also be described best in terms of a change in the chemical nature of the proteins of the syncytium although other factors of importance may obtain. The gradual increase in acidophilia and decrease in basophilia would follow an increase in the total available net positive charge and a decrease in the net negative charge of the protein complex, i.e., an increase in the overall isoelectric point of the protein complex. These changes in the available charge of the syncytial proteins reflect in part the gradual decline in ribonucleoprotein, although presumably they are influenced by other chemical alterations.

Finally, some correlations may be drawn between acidophilia and alkaline phosphatase of the placental syncytium. In the early weeks of development alkaline phosphatase is observed in occasional villi in a thin strip of syncytium subjacent to the brush border (Dempsey and Wislocki, '45). In later development, it extends into the deeper perinuclear cytoplasm of all of the villi. The presence of the highly acidophilic intermediate zone in some of the villi of the young placenta is reminiscent of the distribution of the alkaline phosphatase (compare fig. 1 with fig. 1 of Dempsey and Wislocki, '45). Moreover, in later stages of development, with steadily increasing phosphatase, high acidophilia also invades the innermost syncytial zone. The similarities in distribution of alkaline phosphatase and acidophilia suggest a causal relation between the two.

Fibrin and fibrinoid. Previous investigations of the ground substance of the placenta have emphasized differences in the origin, the morphology, and the staining characteristics of fibrin and fibrinoid. In the present study where no colloidal dyes were used the tinctorial reactions of fibrin and fibrinoid were remarkably alike and no chemical differences were detected. It is theoretically possible to obtain similar staining reactions in two protein complexes the constituent substances of which may differ chemically. Yet, the marked similarity in staining response of fibrin and fibrinoid raises the possibility that these two substances are the same or highly similar. Moreover, their resemblance in staining affinity to a plasma clot suggests a relation to the latter substance. It is quite possible that fibrinogen when clotted under locally different conditions in the placenta may yield two types of clots which are morphologically distinct, in one case being reticulated and fibrillar and in the other homogeneous and compact. In connection with this possibility it is of interest to note that, depending on the clotting conditions, in vitro clotting of fibrinogen yields "fine" or "coarse" clots of differing physical characteristics (Howell, '14; Ferry and Morrison, '47). Fine clots are less fibrillar, physically more homogeneous, trans-

parent, elastic and friable. Their formation is favored by rapid clotting time, increased pH, high ionic strength and temperature of the clotting medium. The reverse of these conditions yields a coarse fibrillar clot with different physical characteristics. Fibrin formation within the placenta may also vary in physical structure depending on local differences in the conditions of clotting — in one instance a coarse clot being elaborated and in the other a homogeneous one. Indeed, different clotting conditions may grade into one another so that both types of clots are formed with no sharp boundary between, an arrangement often observed in sections of the placenta. As the fibrin is elaborated, whether the clot be fibrillar or homogeneous, other substances of the blood and exudates or secretions from surrounding cells or tissues may be caught or carried into its meshes.

SUMMARY

Sections of human placentas of various ages have been stained with an acid dye (orange G) or a basic dye (methylene blue) under controlled conditions and the characteristics of staining of syncytium, fibrin and fibrinoid studied. There are three zones of acid staining and two of basic staining of the syncytium. These are not uniformly present in all villi or in villi of different ages. Young syncytium shows a weak affinity for acid dye which rises gradually to term, the greatest rate of change being in the first 4 months. Basophilia declines throughout pregnancy, its diminution being related to the decrease in the ribonucleoprotein content of the syncytium. The change in staining characteristics of the syncytium suggests a gradual increase in the isoelectric point of the protein complex during ageing.

Placental fibrin and fibrinoid are similar to one another in their staining characteristics and both resemble a plasma clot.

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A STUDY OF THE DISTRIBUTION OF SENSORY AND MOTOR FIBERS IN THE LUMBOSACRAL PLEXUS AND IN THE THORACIC NERVES OF THE CAT

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ONE FIGURE

INTRODUCTION

It has been conclusively shown that the spinal nerves of mammals have within their trunks extensive plexuses, by means of which there takes place a resorting of the fibers both sensory and motor. This has been anatomically demonstrated by gross dissection by Heinemann ('16), Langely and Hashimoto ('17), Compton ('17), Dustin ('18), McKinley ('21), and O'Connell ('36), and also by Marchi degeneration and electrical stimulation in the work of McKinley ('21). These studies have contradicted the older ones of Bardeen ('06), Stoffel ('10) and Kraus and Ingham ('20), which had attempted to demonstrate that groups of nerve fibers to individual muscles were consistently segregated in certain fasciculi, and that these fibers pursued a straight course from nerve plexus to terminal branch. The controversy has been summarized by both McKinley ('21) and O'Connell ('36).

O'Connell ('36) has given the most complete and useful survey of the intraneural plexus in a number of nerves in man. In the unisegmental, intercostal nerves, the fibers of both the motor root and the sensory root, distal and proximal to the ganglion, are arranged in parallel fasciculi without

communications. However, at the junction of anterior and posterior roots an extensive plexus occurs, at which point there is apparently the 1st major sorting out of groups of sensory and motor fibers, both somatic and autonomic. Distal to this coarse plexus the intercostal nerve is composed of a number of parallel fasciculi, among which there are few or no anastomoses. Within each fasciculus, however, there are at intervals fine intraneural plexuses, probably concerned with the final distribution of the fibers in the formation of the various terminal branches of the nerve.

A similar condition is present in the pleurisegmental nerve trunks entering the limbs, except that there are large intraneural plexuses found throughout the length of the nerves, rather than being limited to the junction of the anterior and posterior roots. These additional anastomoses make possible the sorting out of the contributions of the various root segments.

McKinley ('21) studied in the dog the results of partial section of both the common peroneal and the tibial nerves high in the thigh. When stained with the Marchi method, degenerated fibers were found spread diffusely through all lower branches of the semisected trunk, and were not restricted to a few trunks nor to a certain area of each. He likewise found that when certain limited areas of the common peroneal or tibial were stimulated electrically, all muscles supplied by each nerve contracted, rather than only one muscle.

The dog is apparently different from man, in whom the fibers to individual muscles of the arm and leg are localized in certain parts of nerves, according to the operative stimulations of Marie ('15) and Kraus and Ingham ('20). However, only limited exposures of the nerve trunks were possible to these investigators, and their observations could not be well controlled, for they were made on different individuals. Despite the apparent localization of fibers in the nerve trunks which serve certain muscles or muscle groups, their general conclusion that motor fibers pursue a straight course from

plexus to terminal branches is unwarranted on the basis of their evidence.

Thus, there is indirect evidence suggesting that an intermingling of motor and sensory fibers occurs at the formation of the mixed spinal nerve. Moreover, in pleurisegmental (limb) nerves there appears to be a further reshuffling in the nerve trunk of the various root segments. And in all so-called motor nerves there are very fine anastomoses among the fasciculi which probably accomplish the mixture of motor and proprioceptive fibers. The latter agrees with the findings of Sherrington (1894–95) that there is no segregation of afferent and efferent fibers within the trunk of a muscle nerve. It is curious that no one, to the knowledge of the authors, has selectively cut ventral and dorsal roots, and traced the subsequent degenerative pattern of motor and sensory fibers into the plexuses and nerve trunks. This has been done in the present study.

MATERIALS AND METHODS

Five adult female cats were operated upon under sterile conditions using sodium pentobarbital anesthesia. Laminectomies were performed, the bleeding was controlled by Gellfoam, and the desired roots were cut extradurally. This is possible in the cat by carefully picking up the dorsal root proximal to the anglion, and stripping away the dura until dorsal and ventral roots are separated. No degeneration was found later in the uncut root. Dorsal roots were cut distal to their ganglia on the right side of the animals, and ventral roots on the left.

The animals were allowed to live from 17–20 days, the 1st three days of which they received intramuscular injections of penicillin, averaging 25,000 units per day. They were sacrificed under anesthesia by intracardiac perfusion of a potassium dichromate and magnesium sulfate solution, and treated with osmic acid according to the Swank and Davenport ('35) modification of Marchi. The completeness of all operations was confirmed at autopsy.

Blocks of nerves were removed, as shown in figure 1, their orientation being maintained by marking with India ink. In all cases the uncut root was removed and sectioned. Embedding was in paraffin and the sections were cut at 30 micra.

The animals were prepared in the following way:

(a) cat no 2, 6 lbs., operated April 7th, killed April 27th; dorsal and ventral roots cut L5,6.

(b) cat no. 3, 10.75 lbs., operated April 16th, killed May 3rd; dorsal and ventral roots cut L6, 7 and S1.

(c) cat no. 4, 9.75 lbs., operated April 19th, killed May 7th; dorsal and ventral roots cut L6, 7 and S1.

(d) cat no. 5, 8 lbs., operated April 24th, killed May 14th; dorsal and ventral roots cut T6, 7, 8.

(e) cat no. 6, 5.5 lbs., operated June 7th, killed June 25th; dorsal and ventral roots cut L5, 6, 7, and S1.

OBSERVATIONS

Figure 1 is a semidiagramatic drawing of the lumbosacral plexus of the cat to show the points from which the blocks were taken. Their positions were identical on both sides, except for numbers 1, of which the dorsal roots were cut on the right side, and the ventral roots on the left. L4 and S2 were not included in the study or the sketch because their contribution to the lumbosacral plexus was very small or lacking (cf. Langley, 1891). The blocks will be referred to by their numbers in the following description.

1. The uncut roots were removed and sectioned as a control. In no case did they show signs of degeneration. The cut roots were completely degenerated.

2. It was of considerable interest to discover that the two streams of fibers, motor and sensory, became immediately intermingled at the junction of the roots.

3. This block was taken to include the trunk of L5, and its two branches, one to the femoral and one to the obturator nerve. In the common trunk as well as in the bifurcation, motor and sensory fibers were scattered throughout. The common trunk of L5 was chiefly sensory, as was its contrib-

ution to the femoral; the branch to the obturator was mainly motor.

4. This shows the formation of the femoral nerve from L5 and L6. The contribution of L5 is discussed above. The branch from L6 was likewise chiefly sensory, but the relatively few motor fibers were limited to the lateral one-half.

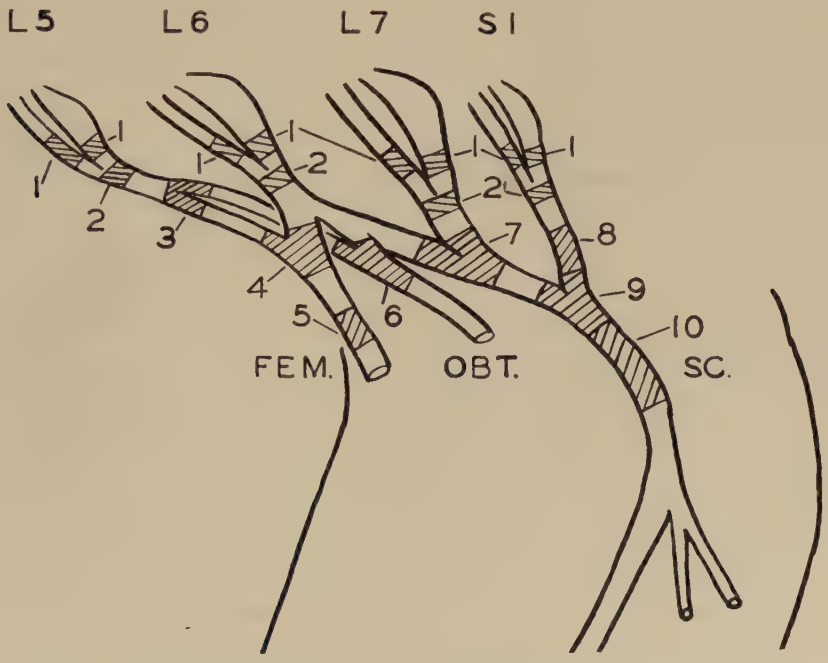

Fig. 1 Semidiagramatic drawing of the lumbosacral plexus of the cat, showing the formation of the femoral, obturator and sciatic nerves from L5, 6, 7, and S1. The blocks removed for this study are cross-hatched and numbered. All removals were identical on both sides, except that the dorsal roots were cut on the right side, and the ventral on the left.

The base of the femoral nerve shows the motor fibers concentrated around the periphery, surrounding a relatively pure mass of sensory fibers. This inner part was the origin of a large cutaneous fascicle.

5. The composition of the femoral nerve was highly variable, and the number of animals examined does not permit

a reliable statement on its divisions. In all cases, however, the rami of L5 and L6 fused to form a common trunk, which soon divided into a number of fascicles, one of which was purely sensory. In the other fasciculi the motor and sensory degeneration was scattered throughout.

6. This block showed the formation of the obturator nerve from L5 and L6. The branch from L5 went entirely to the obturator, none of it extending below into the formation of the sciatic. The branch from L6 divided, part going to the obturator, part to join L7. In cats 2 and 6, in which both L5 and L6 were cut, the motor and sensory degeneration was scattered throughout the obturator nerve. L5 was not cut in animals 3 and 4, and the sensory and motor contributions from L6 alone could be studied. They were limited to the lateral (or dorsal) one half of the nerve, and the obvious deduction is that the undegenerated half contained the fibers from L5.

7. The arrangement of fibers, both motor and sensory, in the combined trunk from L6 and L7 was interesting. Both types of fibers were concentrated peripherally, leaving a central area relatively free of degeneration. This was true not only in the combined nerve, but in the lower parts of the contributing trunks of L6 and L7. The central core was filled with normal fibers of many sizes, and the failure of many of them to show degeneration can be attributed to the uncertainties of the technique. But the constant pattern of peripheral degeneration, plus the fact that the autonomic gray rami were not severed in the operations, suggest that the sympathetic fibers to the hindleg are concentrated in the center of the trunks of L6, L7 and of the sciatic nerve itself *(vide infra)*.

8. The branch from S1 contributing to the sciatic nerve, in contrast to those from L6 and L7, showed both motor and sensory degeneration scattered throughout.

9. This block, taken at the junction of the combined trunk of L6, 7 and S1 to form the sciatic nerve, showed the degener-

ation of motor and sensory fibers concentrated around the periphery of the fascicles.

10. The sciatic nerve, like the femoral, was highly variable in the number and arrangement of its fascicles, and this has been previously shown by McKinley ('21) and by Goldberg ('24). In our preparations, most of the fasciculi in the upper part of the nerve showed the maximal degeneration, both sensory and motor, around the periphery.

In addition, the dorsal and ventral roots of T6, 7, 8 were cut selectively in cat no. 5. Blocks corresponding in position to 1 and 2 in figure 1, were removed as well as several in the main trunks of the nerves. Degeneration of both sensory and motor fibers was scattered throughout the nerves in all of the areas examined.

DISCUSSION

These data confirm histologically certain inferences from gross dissection as to the structure of the spinal nerve trunks, and add other observations which are new. The presence of an intraneural plexus at the point of union of dorsal and ventral roots (O'Connell, '36), receives its explanation in our findings of complete intermixture of motor and sensory fibers at this point. This is true of at least the lower thoracic nerves, and those of the lumbosacral plexus in the cat.

Despite O'Connell's earlier gross findings, such an extensive admixture of afferent and efferent fibers was a surprise, because, in a series of sheep fetuses described by Barron ('43, '44) and by Sprague ('46) and in the rat fetuses by one of the present authors (J. M. S., unpublished), the streams of sensory and motor fibers clearly occupied different halves of the spinal nerves. The posterior rami were formed, in turn, by a few fibers decussating at right angles through the main mass of axons.

Langley and Hashimoto ('17), who dissected a number of nerves in the hind limbs of the cat, dog, goat and man, first suggested that the extensive internal plexuses there present served the purpose of sorting out the contributions of the

various root segments in the main nerve trunks. Our observations show that, despite the intermixture of afferent and efferent fibers just beyond the junction of the dorsal and ventral roots, the total sum of fibers from each segment remains separate from those of adjacent segments for some distance after the formation of a common trunk. This is true in the formation of the femoral and obturator nerves from L5 and L6; the sciatic was not investigated in this respect. That these root segment fibers remain in distinct groups well into the main nerves gives pointed confirmation to the suggestion of Langley and Hashimoto relative to the function of the intraneural plexuses. Our observations support fully the distinction, drawn by O'Connell ('36), between the proximal internal plexus, for the mixture of dorsal and ventral root fibers, and the more distal plexuses, for the sorting out of the contributions of various root segments.

We have also found a marked difference in the position of the somatic motor and sensory fibers in the femoral and obturator nerves and their roots, on the one hand, and the sciatic nerve and its roots, on the other. Degeneration of either motor or sensory fibers in the femoral and obturator nerves was spread thoughout the diameter of the nerve, as well as in the roots entering it. In the trunks (L6, 7) contributing to the sciatic nerve, and at least in the upper part of the nerve itself, both sensory and motor fiber degeneration was concentrated around the periphery, and surrounded a largely intact central area. Since this was a constant finding in 4 animals, which in all nerves showed excellent Marchi preparations, this distribution is not attributed to poor technique. The operations, as performed, did not interrupt the the rami communicantes of the sympathetic system, and the gray rami of L6, 7 and S1 were thus intact. According to Langley (1891, 1894–95) the secretory and vasomotor fibers of the hind leg of the cat leave the sympathetic chain in the gray rami of these trunks. That these rami, although mostly of unmyelinated fibers, contain a considerable number of medullated ones (up to 7.2 micra and above in diameter) is

attested to by the same author (Langley, 1892). Our findings therefore suggest that in the trunks of L6 and L7, and in at least the upper part of the sciatic nerve, the somatic fibers are placed peripherally and the autonomic concentrated centrally. That there is considerable overlap is certain, and the Marchi technique is not precise enough to allow further definition. A sampling of normal fibers in the core of the nerve was measured with a micrometer, and many of them were too large to belong to the sympathetic system. The failure of these to degenerate was due to the uncertainties of the Marchi method, for the roots were completely sectioned and maximum time was allowed for degeneration. The overall, constant picture, however, supports the general conclusion given above.

SUMMARY

The present study of the degeneration of somatic motor and sensory fibers in the cat, after the selective cutting of ventral and dorsal roots of the lower thoracic nerves and those of the lumbosacral plexus, indicates the following:

1. The streams of afferent and efferent fibers, so completely separated in the roots, are immediately intermixed at the root junction in all nerves examined.

2. In the femoral and obturator nerves, at least in their proximal parts, the total fiber contribution of L5 is separated from that of L6. Those of L5 are ventral (medial) and those of L6 dorsal (lateral). Thus, the root segments maintain their independence for some distance in the main nerve trunks.

3. The total motor contribution from L5 and L6 to the obturator and femoral nerves, is not separated in the main nerve trunk from the sensory contribution, but rather the two are intermingled.

4. The somatic motor and sensory fibers in the sciatic nerve, and in its contributory trunks from L6 and L7, are concentrated peripherally. The assumption is made that the postganglionic sympathetic (vasomotor and secretory) fibers are chiefly central in position, since the rami communicantes were not disturbed.

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AN UNUSUAL ANOMALOUS BLOOD VESSEL CONNECTING THE RENAL AND INTERNAL SPERMATIC ARTERIES

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TWO FIGURES

In the course of dissection of a human cadaver a vascular anomaly was discovered. A search of the literature did not reveal a similar case. It therefore seemed worthwhile to describe what appears to be a relatively rare anatomical variation.

DESCRIPTION

The cadaver was that of a white male 57 years of age who died of fractures and internal injuries sustained in an accident.

On the anterolateral surface of the right kidney, opposite the center of the hilum, an artery 1.5 mm in diameter pierced the renal capsule (fig. 1). The vessel arose from an interlobar artery as the latter began to pass between the pyramids of the kidney (fig. 2). At the inferior border of the kidney it turned sharply medialward. When it reached the psoas major, it coursed caudally over the anterior surface of this muscle, between the fused layers of renal fascia, and anastomosed with the right internal spermatic artery where the latter crossed the anterior surface of the right ureter 6 cm above the abdominal inguinal ring.

Inferior to the kidney the anomalous artery gave off several branches which anastomosed with perirenal vessels. It gave rise to minute branches which supplied both the retroperitoneal tissue along its course and the portion of the ureter adjacent to the internal spermatic artery.

The internal spermatic artery arose from the aorta at the normal level, a little inferior to the renal artery; it passed laterally, dorsal to the inferior vena cava, and then descended along its usual course.

The right kidney was in the normal position, its superior extremity being at the level of the 12th thoracic vertebra and its inferior extremity at the lower margin of the 3rd lumbar vertebra. The surfaces were smooth and the dimensions were within the normal range, 12.5 cm in length, 5.5 cm in width, and 3.5 cm in thickness.

The left kidney was in a normal position, a little higher than the right, but was distinctly lobulated. Its anterior surface was divided into 6 lobes; the posterior surface was smooth.

Two other less unusual anomalies of the vascular system were also found. A large accessory hepatic artery branched from the left gastric artery. This variation occurs in about 17% of cadavers (Adachi, '28). The right spermatic vein emptied into the right renal vein (fig. 1), a not uncommon occurrence.

DISCUSSION

Descriptions of only two anomalies which seem to be related to the case described in this paper were found in the literature.

Eustachius, who in 1564 made the first anatomical copper-plate engravings, illustrated a renal arterial anomaly. Lancisi, who published the engravings with a commentary in 1714, referred to the anomaly as *lusus naturae*. It was found in a male cadaver, presumably an adult. The engraving indicates that the right kidney was in a normal position. A slender artery emerged from its anterior surface at a point slightly lower than the origin of the anomalous vessel described in the present paper and descended to anastomose with what was probably the right hypogastric artery, the junction occurring close to the origin of the latter. Lancisi referred to the anomalous vessel as if it were a supplement to what he called the "draining arteries" of the kidney and mentioned that

the latter are occasionally more slender than the one described. No mention was made as to what connection the anomalous artery had within the kidney.

Another anomaly, described by Dawson and Reis ('22), appears closely to resemble the anomaly with which the writer is concerned. In a full-term fetus they discovered a right ovarian artery which arose from the renal artery within rather than outside the kidney. This ovarian artery was formed by the union of three branches from the interlobar renal arteries. It emerged from the inferior pole of the kidney, and its relations caudal to the kidney were practically normal.

The anomalous artery discussed in this paper could undoubtedly be a homologue of one of the three interconnecting branches between the renal and ovarian arteries mentioned by Dawson and Reis. If this interpretation is correct, the anomalous artery of the present paper and the interconnecting branches of Dawson and Reis are quite significant in substantiating the Bremer ('15) theory of the origin of the renal and internal spermatic arteries.

Although Bremer ('15) is usually regarded as the originator of the theory, Felix in 1912 described the presence of three sets of segmented arteries. He referred to them as the mesonephric segmented and viscero-ventral and dorsal arches and described them in an 18 mm human embryo. Felix also described completely the plexus of mesonephric blood vessels uniting the developing gonad with the metanephros and called this network *rete arteriosum urogenitale.*

Bremer stated that the three sets of segmented arteries unite to form a periaortic net from which the suprarenal, renal, and ovarian or spermatic arteries develop and that any one of several paths among these interconnecting channels may become a permanent vessel. Therefore, multiple renal arteries thus represent the persistence of several of the horizontal sprouts of the aorta. Bremer added that if the renal artery is connected in any way with the mesonephric plexus, it would be common to find, as an anomaly, the sper-

matic artery as its branch. Since his paper, this type of anomaly has been observed quite frequently. The vessels joining the renal artery to the internal spermatic, ovarian, or hypogastric artery as described in the present paper and in that of Dawson and Reis ('22) and as illustrated in Eustachius' plate (1714), clearly support Bremer's theory regarding an early periaortic net.

Bremer, disagreeing with other investigators, believed that there are no "late" (i.e., postnatally developed) branches. A condensation of mesenchyma around a vessel to form its coat, according to Bremer, should prevent any further out-growth of endothelium to form new branches. He stated that such a mesodermal wall, in a layer three or 4 cells thick, is found in 100 mm human embryos.

It must be recognized that the presence of a very slightly differentiated capsule around an organ will protect it from further in-growth of blood vessels. The renal capsule is distinct in man at 13.0 mm, though not completely formed until much later (Wilson, '12).

The possibility was considered that the anomalous artery might have been acquired post-embryologically by the enlargement of small arteries normally found in the region of the anomalous vessel. The artery might have originated within the kidney as a very enlarged perforating capsular artery arising from an interlobular artery. If so, the remaining portion, by means of normal anastomotic connections, could then have been an enlarged perirenal vessel which supplies the perirenal fat body, and branches of the renal and internal spermatic arteries which supply the ureter and retroperitoneal tissue.

The artery distinctly arose from an interlobar artery, however, 1.5 cm before the interlobar gave rise to an arcuate artery. Furthermore, careful examination of the renal and internal spermatic arteries, as well as the aorta, failed to reveal an obstruction which might require collateral circulation. Although abundantly supplied with vessels, chiefly

by the hilar route, the kidneys are notoriously incapable of developing an adequate collateral circulation (Pick and Anson, '40). A theory of postnatal acquisition of the anomaly, therefore, scarcely seems tenable.

SUMMARY

1. In an adult male cadaver an anomalous artery was found to arise from an interlobar artery within the right kidney, pierce the anterolateral surface of the renal capsule, descend inferiorly over the anterior surface of the psoas muscle, and terminate by an anastomosis with the right internal spermatic artery 6 cm above the level of the abdominal inguinal ring.

2. Bremer ('15) recognized the presence of a periaortic plexus made up of the anastomosis of three sets of segmental arteries arising from the primitive aorta, and assumed that it is possible for any one of these segmental vessels and their interconnecting branches to persist. It seems plausible that in the present case the anomalous artery was a retained interconnecting vessel between the renal and internal spermatic arteries.

3. The possibility that the anomalous artery arose postembryologically was considered untenable.

ACKNOWLEDGMENTS

The author wishes to thank Mr. B. H. Griffith, Dr. B. L. Truscott, Dr. T. R. Forbes, and Dr. W. U. Gardner for suggestions and criticisms.

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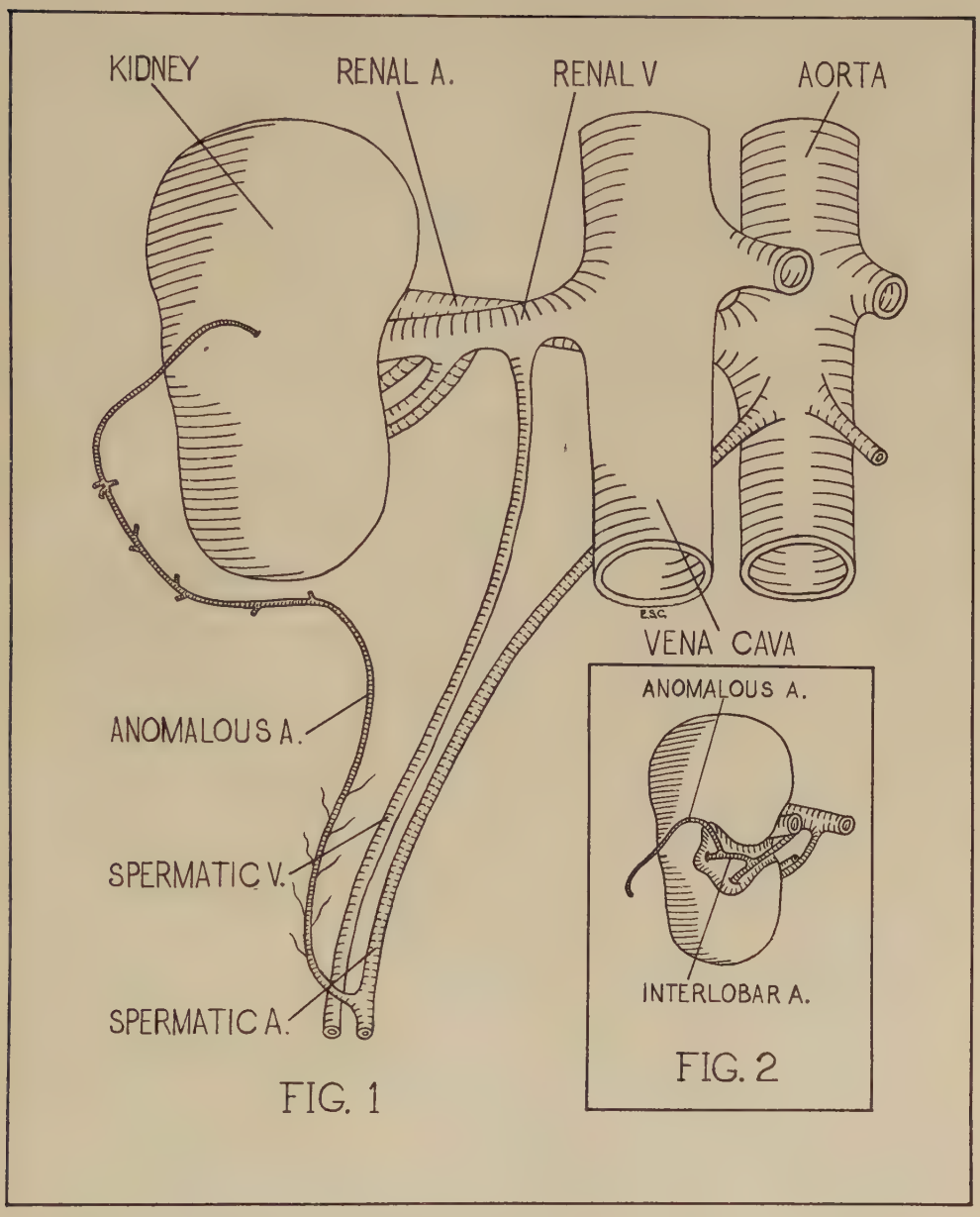

1 Shown are the right kidney and its blood supply, including the anomalous spermatic vein, the internal spermatic artery, and the anomalous artery.

2 A portion of the right kidney has been removed, showing the anomalous artery arising from an interlobar artery within the kidney.

THE HISTOLOGIC STRUCTURE OF THE LYMPH NODE CAPSULE AT THE HILUM

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FIVE FIGURES

In a previous study of lymph node hyperplasia the writer ('48) observed that the structure of the capsule in the hilar region differed from the accepted morphologic descriptions. In both normal and hyperplastic nodes of hamsters, rats, and calves, a compact capsule of varying thickness enveloped the cortex, but at the hilum the medulla was covered only by a less dense tissue which filled the hilar depression.

The literature reveals that Heudorfer ('21) in his morphologic investigation of lymph nodes of cats and dogs had described the capsule as a compact structure which merged with a voluminous mass of connective tissue at the hilum. Such tissue, which also bounds the periphery of the medulla he designated as the "Hilusstroma von His" in acknowledgment of the original observer. Its structural features are clearly expressed in Heudorfer's statement: "Der Hilus welcher gleichsam eine Einstülpung der Kapsel darstellt, besteht aus Bindegewebe von vergleichweise lockerem Bau. Nicht selten sind ihm zahlreiche Fettzellen eingelagert." Unfortunately the schema of the lymph node contained in his report, and which has been copied by many subsequent authors, shows the hilum to be filled with a dense, thickened continuation of the cortical capsule.

For some unknown reason, however, Heudorfer's contribution has been either overlooked or repudiated by most Am-

erican authors of current textbooks of histology. Bailey's Textbook of Histology, eleventh edition, states: "The capsule consists of rather closely packed bundles of fibrous connective and scattered elastic fibers, which are more numerous in its inner layer. A few smooth muscle cell fibers can be found in the capsule around the points of entrance and exit of lymphatic vessels. At the hilum there is a depression where the capsule is thickened and extends deep into the node." According to Maximow and Bloom's Textbook of Histology, fourth edition: "At the hilus the capsule is greatly thickened and may extend for some distance into the medullary portion of the node." In other texts (Bremer, Jordan, Cowdry) such details pertaining to the nature of the capsule are not mentioned. Hellman ('30) in Mollendorff's Handbuch der Mikroscopischen Anatomie des Menschen is cognizant of Heudorfer's findings and adds that the projections of the medulla extend into the connective tissue of the hilum.

In view of the discrepancies noted above it was felt that a critical re-examination of the structural relationships of the lymph node hilum was warranted. The material used consisted of: (1) normal and hyperplastic nodes of hamsters, rats, and calves which were prepared and studied by the writer in his previous work; (2) slides of normal human lymph nodes stained with hematoxylin-eosin or hematoxylin-picrofuchsin; (3) slides stained with hematoxylin-eosin of pathologic human nodes which had been extirpated for biopsy purposes. The writer thanks Dr. O. F. Kampmeier, Chairman, Department of Anatomy, University of Illinois College of Medicine, Chicago, Illinois, and Dr. G. A. Bennett, Chairman, Department of Pathology of the same institution, for placing the human materials at his disposal.

This comparative study demonstrated again the importance of materials sectioned serially with proper orientation. To obtain the true morphologic relations of the structures in the hilar region it was necessary to examine several consecutive sections which passed through the greatest area of the hilum.

OBSERVATIONS

Rats. The capsule of the normal lymph node is a thin but compact structure which covers the peripheral margin of the cortex and encloses the subcapsular sinus. Composed essentially of fibroblasts and collagenous fibers, it measures approximately 10–15 microns in thickness. At the hilum the capsule suddenly disappears by merging insensibly with the areolar tissue which fills the depression. Figure 1 shows an area through the hilar region of a popliteal lymph node of an adult rat. There is no indication of a compact enclosure over the external margin of the medulla (*m*). From the latter, lymphocytes and free reticular cells migrate diffusely into the hilum and as a result the marginal contour of the node here is quite indistinct. Slides stained with hematoxylin-picrofuchsin fail to demonstrate any muscle fibers in the loose tissue of the hilum.

Hamsters. A fibrous capsule somewhat thinner and delicate than that of the rat lymph node extends over the entire cortical surface. This again blends with the areolar tissue of the hilum. The peripheral infiltration of cells from the medulla is not as marked as in the rat.

Calves. The large lymph nodes possess a dense capsule which consists mainly of coarse collagenous fibers and some smooth muscle cells. Its average thickness in the cortical region is about 75–100 microns but these measurements dwindle to about one-fourth as the hilum is approached. At the latter site the thinned capsule breaks up into interrupted strands which mingle with the abundant but loose tissue of the hilar depression. Such features of the hilum in a normal mesenteric lymph node is illustrated in figure 2. The capsular remnants (*x*), which may be either few and prominent or numerous and fine, retain their structural components and stain similarly to the intact portion of the capsule (appearing at the left of the figure). Examination consecutively of serial sections reveals that small efferent lymphatics pass through the interstices between the capsular islands and empty into a

large collecting trunk which is located more peripherally (*e*). The blood vascular system of the node shows a similar arrangement. The gaps are also the means by which lymphocytes and reticular cells emigrate diffusely into the hilum. As a matter of fact, marginal protrusions of the medulla in this manner often occur.

Except for the region near the border of the medulla, which contains segments of the capsule and infiltrated cells, the tissue packing the hilum is typically areolar. Only rarely, however, delicate threads of smooth muscle are seen intermingled with the collagenous and reticular fibers. A word may be said here about the trabeculae which arise from the hilum and pass centripetally into the medulla, since the abundance of the trabecular framework in the calf node facilitates such observation. In a typical arrangement the majority of the trabeculae course inward from the depth of the hilum along with the arterial and venous branches, while a few are independent of the vascular tree. At their origin in the apex of the hilum the trabeculae are of areolar structure and become compact and dense only after penetrating the medullary parenchyma. Scattered smooth muscle fibers are found in the intra-nodal portions. Unlike the relatively indefinite enclosure around the medulla, the arrangement over the cortex of the node presents a striking contrast (fig. 3). The perinodal areolar tissue is entirely devoid of cells infiltrated from the interior of the organ.

Human nodes. The capsule of a normal lymph node consists predominantly of white fibrous connective tissue. When compared with that of the calf node, it is less dense and thinner. Filaments of smooth muscle are found here and there among the fibroblasts. There is a gradual blending of its outer surface with the surrounding perinodal tissue. In the cortical region the capsule is approximately 25–50 microns in thickness and thins out towards the hilum.

The coalescence of the capsule with the tissue occupying the hilar depression, an arrangement which obtains in specimens of rats, hamsters and calves, is a typical feature also in

the human node. The collagenous fibers of the capsule are incorporated so intimately with those of the hilar stroma that it is impossible to detect any traces of capsular remnants. Figure 4 depicts a central portion of the hilum of a normal cervical lymph node from an adult man. The marginal contour of the medulla is interrupted by numerous small lymphatics which pass peripherally towards the large plexus of efferent vessels (*e*). A definite enclosure comparable to the cortical capsule is not present and the medulla sends irregular cords of lymphatic tissue (*l*) into the hilum. The connective tissue of the hilum is typically areolar, both with respect to its consistency and its structural components. Smooth muscle fibers, aside from those contained in the walls of hemal and lymphatic vessels, are not found. The trabeculae passing inward are few and slender. As in the node of the calf, they originate as condensations of the areolar tissue of the hilum.

The examination of hyperplastic nodes which were removed surgically merely substantiates the findings in normal human specimens. Even in those cases where fibrotic changes of the entire stroma had occurred, the connective tissue of the hilum maintains its loose structure. Figure 5 represents an area through the hilar region of an adult inguinal node. The cortex, a part of which appears at the upper right is surrounded by a fibrosed capsule (*k*). The latter continues into the node as a trabecula at (*t*). The medulla, (*m*), on the other hand, is surrounded by the areolar tissue of the hilum (*h*) which, in this case, is rather heavily infiltrated with lymphocytes and free reticular cells.

DISCUSSION AND SUMMARY

The observations presented here indicate that in the lymph node the capsule exists as a solid covering only over the outer margin of the cortex. It is continuous at the hilum with the areolar tissue of the hilar depression. The latter tissue serves as a support around the vessels and the nerves of the node and at the same time delimits the peripheral margin of the medulla. In the rat, in the hamster, and in man,

the identity of the cortical capsule is lost as it coalesces with the hilar connective tissue, but in the node of the calf small, discrete capsular islands remain dispersed in the hilum.

These findings, in addition to confirming the conclusions of Heudorfer ('21), demonstrate that a capsule in the true sense of the word does not exist around the medulla. In acute hyperplasia most of the peripheral enlargement of the node occurred by the protrusion of medullary tissue into the loose stroma of the hilum (Furuta, '48).

The accepted concept that the fibrous capsule of the lymph node is thicker at the hilum, requires qualification. To consider the connective tissue of the hilum as an integral part of the capsule seems justified since the two are intimately connected and constitute a complete enclosure around the organ. The "thicker" portion which covers the hilar region should be defined as consisting not of dense fibrous but of areolar connective tissue.

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PLATES

PLATE 1

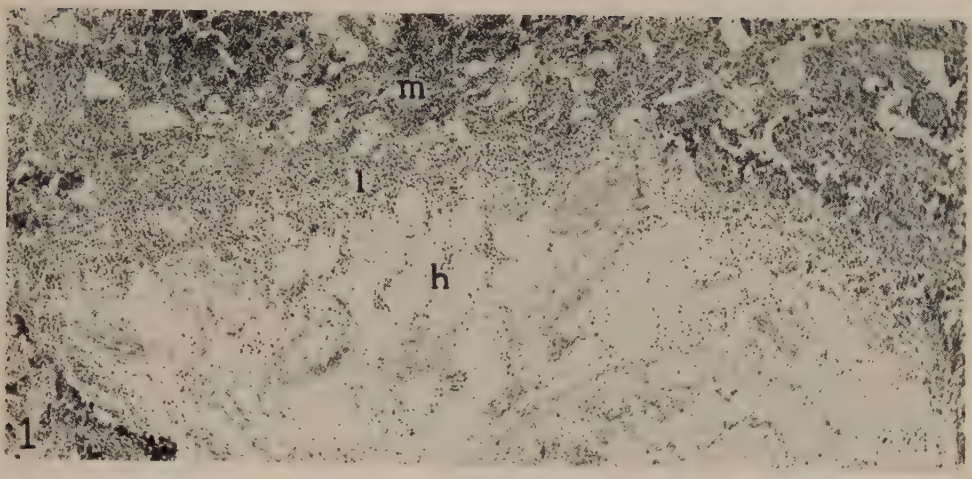
EXPLANATION OF FIGURES

Figures 1–5, inclusive, are unretouched photomicrographs.

1 Section through the hilum of a normal popliteal lymph node of an adult rat. × 95. *m*, medulla; *h*, areolar tissue of hilum; *l*, lymphocytes and free reticular cells infiltrated into hilum from medulla. Note the absence of a solid enclosure around the margin of the medulla.

2 Section through the hilum of a normal mesenteric lymph node of a calf. × 100. *k*, intact capsule; *x*, islands of capsular tissue; *e*, efferent lymphatic vessel; *h*, areolar tissue of hilum. Note the infiltration of cells peripherally into the stroma of the hilum.

3 Section through the cortical margin of the node shown in figure 2. × 100. *k*, capsule; *c*, cortex; *p*, perinodal areolar tissue. Note the thicker capsule and the absence of infiltrated cells into the perinodal tissue.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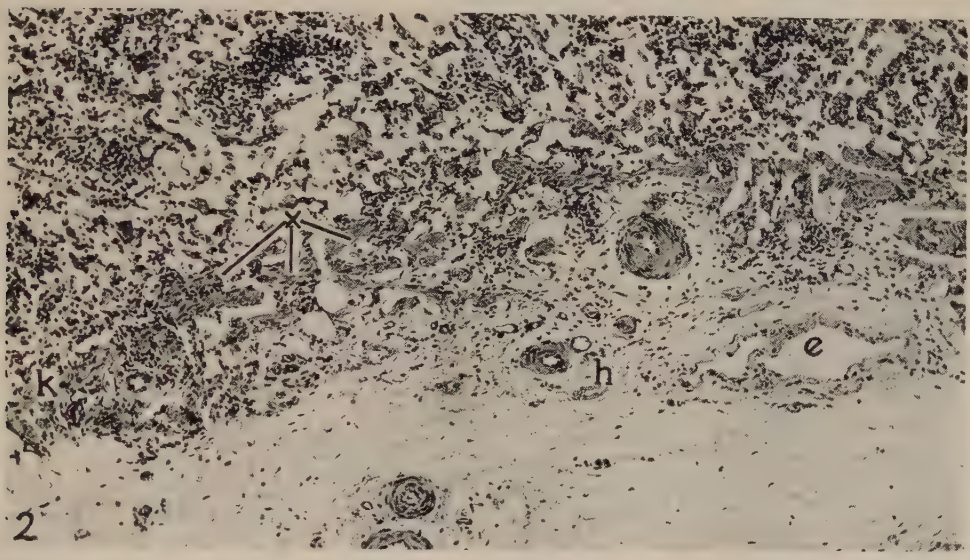
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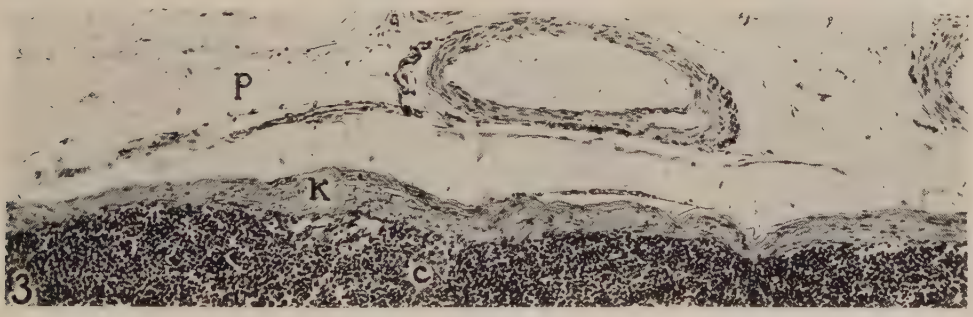
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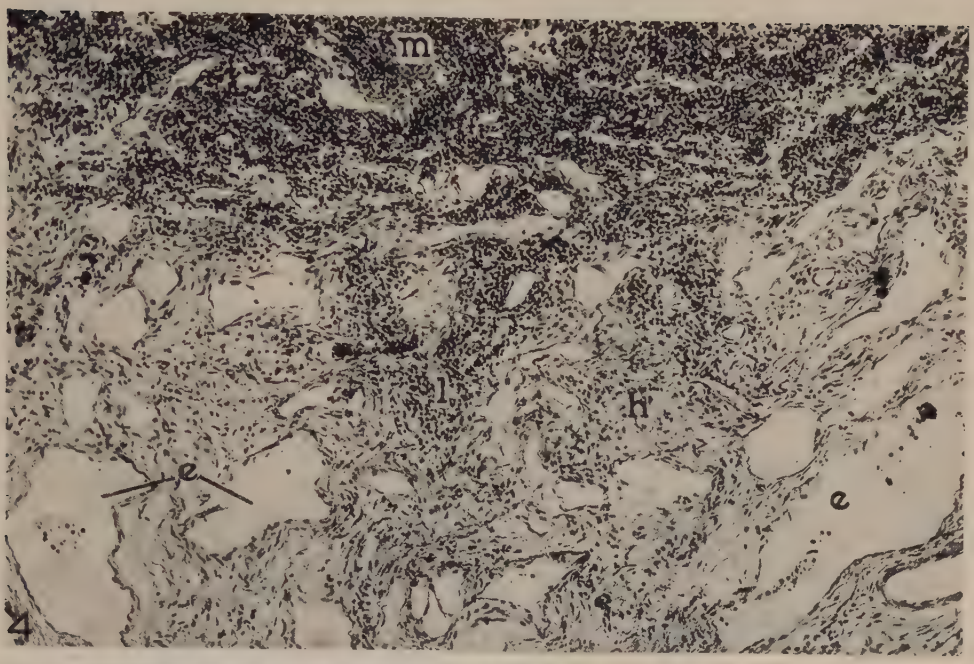
PLATE 2

EXPLANATION OF FIGURES

4 Section through the hilum of a normal cervical lymph node of an adult man. × 100. *m*, medulla; *l*, lymphocytes and reticular cells infiltrated from the medulla; *e*, efferent lymphatics; *h*, areolar tissue of hilum.

5 Section through the hilum of a human inguinal lymph node, chronic lymphadenitis. × 100. *m*, medulla; *h*, areolar tissue of hilum; *e*, efferent lymphatics; *c*, cortex; *s*, subcapsular sinus; *k*, capsule; *t*, trabecula. Note that a thick, fibrosed capsule surroundes the cortical portion of the node, but that the medulla lies in direct contact with the loose tissue of the hilum.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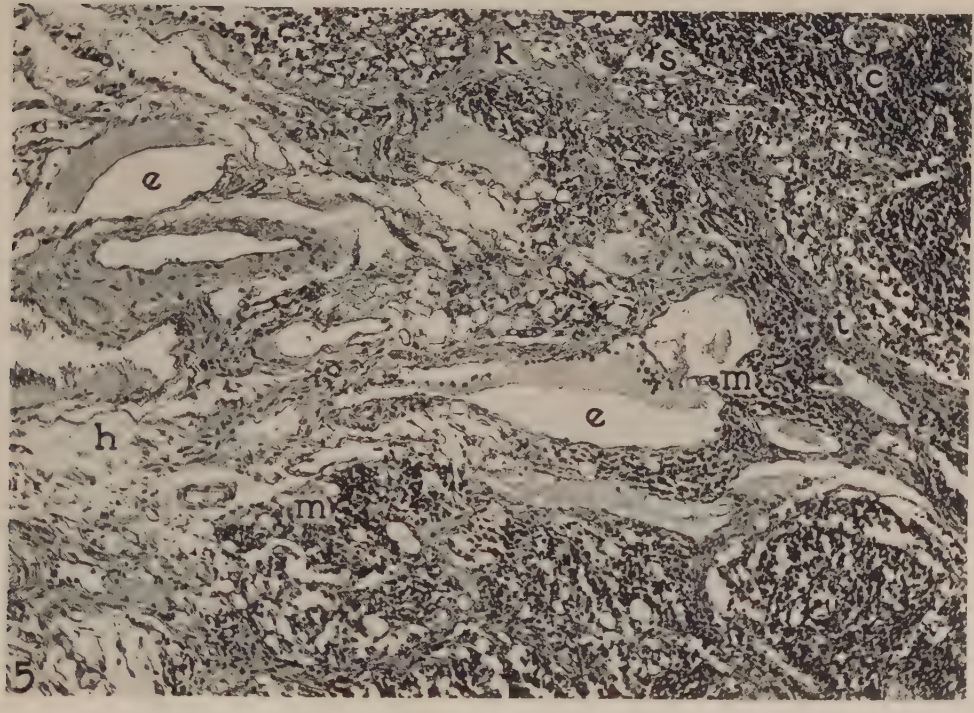
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DEVELOPMENT OF BONE FROM TRANSPLANTED MARROW IN MICE[1, 2]

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SIX FIGURES

Atttempts to analyze the factors responsible for the development or repair of bone have been numerous. The effects of sex hormones have been reviewed by Gardner and Pfeiffer ('43), and the influence of the hypophysis has been shown by Silberberg and Silberberg ('37, '43) and Ray, Evans and Becks ('41). In 1941 it was demonstrated by Bloom, Bloom and McLean that the marrow reticulum cells differentiate into osteoblasts during the formation of medullary bone and become osteoclasts during bone resorption. This behavior suggested that bone could be produced from the reticulum cells of the marrow in the absence of both endosteum and formed bone, if the proper environment were present.

Ollier (1867) was apparently the first to transplant marrow to see if it would form bone. He failed with homoplastic transplants in the rabbit, dog, cat, fowl and dove. However, Goujon (1869) claimed some success with young rabbits and fowl. Baikow (1870) repeated Ollier's experiments and like-

[1] This investigation was aided by grants from the James Hudson Brown Memorial Fund of the Yale University School of Medicine, The Anna Fuller Fund and the U. S. Public Health Service.

[2] Part of this material was presented at the Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing. Transactions of the sixth meeting, February 11–12, 1944. Transactions distributed by the Josiah Macy, Jr. Foundation, New York, N. Y., pp. 88–92. Also part of this material was presented at the meeting of the American Society of Zoologists held in Cleveland, Ohio, September 12–14, 1944. See abstract, Pfeiffer, C. A., 1944. Development of bone from transplanted marrow. Anat. Rec., vol. 89, pp. 534–535.

wise failed with homoplastic transplants, but was successful in obtaining bone growth in 14 of 28 autotransplants. Maas (1877), on the other hand, denied that the marrow has any osteogenic properties. Bruns (1881) also failed to obtain bone in homoplastic transplants of bone marrow in the rabbit, chicken and dog but, like Baikow, was successful with autotransplants in both pups and full grown dogs in 12 out of 19 cases. Chiari ('12), in a study of the capacity of transplanted bone marrow of the rabbit to produce blood cells, observed small bone spicules but did not state whether they were formed from the transplanted bone marrow or were simply transplanted with it. Miyauchi ('14) and Bull ('28) were able to get bone to grow in autoplastic transplants of bone marrow in young rabbits. Levander ('40) made subcutaneous autoplastic bone marrow grafts into both young and adult rabbits and obtained bone formation but reported that it was not produced from the marrow itself but from the mesenchymal tissue around it which had been induced to form bone by some osteogenic substance in the marrow.

The fact that endosteal bone formation is easily stimulated in mice by estrogen (Gardner and Pfeiffer, '38a) suggested that the mouse might prove a favorable mammal on which to investigate the osteogenic potentialities of the marrow reticulum cells. It was therefore decided to remove the marrow from its normal environment in the mouse to sites which differ in their resistance to grafting and in which the grafted tissue can be easily localized. The anterior chamber of the eye and the testis were chosen as places where the resistance to grafting is low, and the subcutaneous tissue of the ear was chosen as a position where resistance to grafting is high. In all of these places the grafts can be readily localized. The present paper deals with the response of the marrow in these grafting sites.

MATERIAL AND METHODS

Mice of the Strong ('36) A strain were used in these experiments because, in addition to the advantage that the eyes

and skin lack pigment, this strain has been used extensively for other experiments and therefore its response to experimental procedures is well known to the author. Bone marrow implants were made (as shown in table 1) into 87 mice, of which 27 were females, 40 were normal males and 20 were castrated males. All animals were 90 days of age at the start of the experiment. They were given Purina fox chow and water ad libitum. The fox chow was occasionally supplemented with calf meal.

TABLE 1

Bone formation from marrow transplanted into mice of the A strain

NO. OF ANIMALS	SEX	ESTRADIOL BENZOATE WEEKLY	DAYS GRAFT IN HOST	GRAFTING SITES LOCATED	NUMBER OF GRAFTS CONTAINING BONE
		μg			
A. Bone marrow transplanted to the anterior chamber of the eye					
7	♀	16.6	30	4	4
10	♂	...	60	4	3
10	♂	...	60	8	6
10	♂	16.6	60	5	5
10	♂	16.6	60	6	5
B. Bone marrow transplanted into the testis					
10	♂	16.6	70	8	6
10	♂	...	70	9	8
C. Bone marrow transplanted subcutaneously into the ear					
10	♀	16.6	70	?	..
10	♀	...	70	?	..

Since the A strain of mice has been extremely well inbred, grafting from one animal to another is as effective as autografting. Therefore, the donor animal was sacrificed. The femurs were dissected out, and the diaphysis was split carefully, to avoid the introduction of fragments of bone into the marrow cavity. Marrow was removed under a dissecting microscope from the central portion of the marrow cavity, using care not to remove the endosteum or any spicules of bone. The marrow was placed in sterile normal saline and

adjusted to the proper size for transplanting. Because of the small size of the mouse's eye, the marrow was introduced by means of a 27 gauge needle on a $1cm^3$ tuberculin syringe. A very small fragment of marrow was drawn into the needle with normal saline, and the needle was introduced into the eye through a nick that had been made in the cornea near the scleral-corneal junction. This nick allows the escape of the fluid from the anterior chamber. Care had to be used not to introduce saline in quantities that would float the transplant out through the corneal incision. The author later found that the small fragment of marrow could be introduced by a pair of very fine curved forceps somewhat more effectively than with the hypodermic needle.

The hosts were anesthetized with ether just to the point where the eye reflex was no longer present. Then, with the animal lying on its side, the eye was protruded by pressure of thumb and forefinger. The eye was stabilized by grasping the connective tissue of the posterior part of the eyeball with a mosquito clamp in such a way that the weight of the clamp as the handles rested on the operating board put tension on the eye ball. The nick in the cornea at the scleral-corneal junction was made by sliding an iris knife over the top of the mosquito clamp. The marrow was then put into the anterior chamber and moved into the desired position by a slight pressure on the cornea with the handle of the forceps.

The transplants into the ears were made according to the technique of Hill ('37). In all cases the marrow was introduced with fine forceps. A larger fragment could be introduced into the ear than into the eye. The incision was made near the base of the ear, and the skin was separated from the subcutaneous tissue from this point to the tip of the ear. The graft was placed over a blood vessel near the tip of the ear. Marrow was implanted into the testis in the following manner: The testis was exposed by making a mid-line suprapubic incision, pulling the testis up into the abdominal cavity and bringing it to the exterior. A nick was then made in the tunica albuginea at the posterior pole of the testis. The tes-

tis was held in position by grasping the gubernaculum with forceps, and the piece of marrow was inserted with fine curved forceps under the tunica albuginea and between the seminiferous tubules. Fragments of marrow were often removed directly from the marrow cavity of the femur to the testis without being placed, or further fragmented, in physiological saline. Only the right testis was used. By using a standard position for insertion, the grafts were much easier to locate for sectioning.

Most of the animals were autopsied at either 60 or 70 days after the marrow had been transplanted, and all the areas where transplants had been made were prepared for histological study. The testes and ears were preserved in Bouin's fluid since the developing bone could be decalcified simply by extending the time the tissue was left in this fluid. These tissues were sectioned at 8 μ and stained with Erhlich's hematoxylin and triosin. Most of the grafts into the anterior chambers of the eyes were fixed in formalin and stained with Dominici's combination (eosin-orange G and toluidine blue) to study the hemopoietic nature of the marrow.

RESULTS

Transplantation of bone marrow into the anterior chamber of the eye. In preliminary experiments, female mice were used as hosts since it was thought it would be necessary to have estrogen present in order to obtain good bone growth from marrow transplants. All of these females received 16.6 μg of estradiol benzoate [3] weekly. The grafts were removed after 30 days. Upon gross examination, it was seen that 4 of the 7 mice had good grafts in the eye. Fairly large pieces of marrow had been transplanted, and the graft usually filled most of the anterior chamber. Histological study of the grafts revealed that the marrow reticulum had developed compact bone which was laid down in a relatively thin layer over almost the entire under surface of the cornea (fig. 1).

[3] The estradiol benzoate was supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk.

This response gave the erroneous impression that it is necessary to have a somewhat rigid surface for bone to form along. However, as will be seen in later experiments, the configuration obtained was due to the fact that in attempting to graft relatively large amounts of the bone marrow, it became dispersed throughout most of the anterior chamber of the eye.

To determine whether estrogen is essential to the formation of bone from marrow transplanted to the anterior chamber of the eye, 20 normal males and 20 castrated males were used as hosts. Half of each group received 16.6 μg of estradiol benzoate weekly; the others received no further treatment. All were autopsied after 60 days. Bone formed from the marrow transplant in both intact and castrated males, whether or not estrogen was injected (table 1). The grafts did not take as well in the normal as in the castrated males, but when bone was present in the eyes of normal males, it was as well developed as when grown in the castrated mice.

From histological sections it appears that the configuration of the newly formed bone is determined by the position of the marrow transplant in the anterior chamber. Each fragment of growing bone is surrounded by a layer which has the characteristics of an endosteum. Lymphocytes are usually found around the outside of this layer. Plasma cells are also occasionally seen, but fibroblasts and connective tissue are relatively rare in this area. The "endosteal layer" includes osteoblasts and occasionally osteoclasts, indicating that both bone deposition and bone resorption are taking place. Most of the bone fragments are well organized into compact bone (fig. 3). The configuration of the bone usually almost completely precludes its having developed from a bone spicule. No marrow graft could be identified which did not contain developing bone. A few areas of old inflammation and damage to the iris were found which indicated where a graft had been placed which did not grow. One definite factor in the low incidence of grafts is that if the lens is damaged the whole eye becomes opaque. This interferes with the normal

circulation and transforms the eye into an area with high resistance to grafting.

Transplantation of bone marrow under the tunica albuginea of the testis. To test whether there might be something about the anterior chamber of the eye that stimulates the formation of bone from marrow that has been transplanted into it, the effect of another site where the resistance to grafting is low was investigated. For this purpose the testis was chosen since grafts may be made into it more easily than into other areas of low resistance to grafting such as the brain. Twenty male mice were used, of which half received 16.6 μg of estradiol benzoate weekly, and half received no hormone treatment. When the animals were autopsied 10 weeks later, the presence of a graft could not be detected grossly. Therefore, the entire posterior half of the testis was sectioned. A higher percentage of takes was obtained here (table 1) than in the grafts into the eye, presumably because of the greater ease of making the implants. Again the injections of estrogen did not increase the development of bone. If anything, the estrogen was somewhat deleterious since it caused a slight inhibition of the testis.

The grafts in the testis differed histologically from those in the eye, both in the configuration of the developing bone (compare figs. 2 and 4) and in the fact that connective tissue fibers were more numerous. Many small isolated pieces of bone were found in the testis. The smallest were often distributed along the path taken by the forceps in introducing the marrow (fig. 5), and presumbably developed from small fragments of marrow which rubbed off the forceps. These pieces of bone showed the same type of development, however, as did the bone which formed in the anterior chamber of the eye.

Interstitial cells of Leydig were present in the intertubular spaces around the edge of the grafted material but were never found in the connective tissue of the graft. Sometimes a few segments of the seminiferous tubules around the graft showed damage, but most of them were normal. Cells of

the myelopoietic and erythropoietic series were not present. Plasma cells were very numerous in all the grafts. They presumably formed from the reticulum cells of the marrow transplant. It seems evident that at least some of them transformed into fibroblasts, since all stages between plasma cells and fibroblasts were readily seen (fig. 6). The latter then formed collagenous fibers and were presumably the source of the connective tissue which made up the major part of the graft that was not occupied by the developing bone. There was no evidence that the plasma cell is an intermediary stage in the transformation of reticulum cells into osteoblasts. The formation of osteoblasts at the time these grafts were studied was not great enough to allow one to make a definite statement either way on this matter. Lymphocytes were numerous in certain grafts but were not always present. They usually occurred near a piece of developing bone. They were presumably a result of the grafting but apparently took no part in the formation of bone. Bone development did not seem to progress as rapidly in the testis as it did in the eye. The osteoblastic reaction was not as great.

Transplantation of bone marrow subcutaneously into the pinna of the ear. The ear was chosen as a transplantation site since besides being a region where the resistance to grafting is high, it is one in which the transplanted material can be easily localized and can be observed with the aid of transmitted light. At no time was there any evidence that the graft had taken. The ears of a few of the animals were attacked by the cutaneous form of infectious ectromelia, but in only two cases was the grafting site involved. Great care was taken in placing the grafts so that the area could be prepared for histological study. The grafting site was removed 70 days after transplantation of the marrow and sectioned serially. In no instance was any tissue found which could be identified as having been derived from the grafted material. It is to be expected that the myeloid cells would be absent since they were not found in the more favorable grafting sites. However, the reticulum cells were also absent, and it seems probable

that they become tissue wandering cells and migrated from the area.

DISCUSSION

It has been seen in the above description that bone developed in marrow that had been transplanted to the anterior chamber of the eye and between the seminiferous tubules of the testes of mice. Because of the small size of the bones of mice, it is, of course, difficult to be certain that no spicules of bone or fragments of endosteum were transplanted with the marrow, even though only the femur was used as a source of the transplanted material. However, it seems improbable that spicules of bone, if they were present, were the source of the bone that formed in the grafted marrow. Experiments on the transplantation of bone have indicated that it is necessary to transplant rather large pieces in order to get a take and that all small pieces of transplanted bone soon become necrotic and are resorbed. Levander ('40), who has reviewed this subject, is convinced that bone spicules do not grow if they are transplanted with bone marrow.

Several conditions seen in the histological preparations of the grafts made in the present study strongly argue against the possibility that the developing bone originated from transplanted endosteum or bone spicules. First, is the configuration of the developing bone in the anterior chamber of the eye, when the bone was injected in normal saline suspension through a 27 gauge needle. Although fairly large amounts of marrow were sometimes implanted by this means, they became greatly fragmented by being pulled through the needle into the syringe and then forced out again. With good retention, this material was dispersed as a fine cloud throughout the anterior chamber. The newly formed bone developed as a thin plate over the posterior surface of the cornea and covered much of the anterior chamber. It seems unlikely that this configuration would have occurred if it had developed from a fragment of bone or from the relatively firm endosteum, since in the experiments where the marrow was inserted in a

compact piece, bone developed only in the region where the graft was located. The second condition is that a good bone marrow graft was never found that did not contain at least one area of active bone formation. This fact suggests that the healthy, well established condition of the marrow grafts had made it possible for them to produce bone, since it seems improbable, with the care taken in obtaining the marrow used in these experiments, that spicules of bone could have been transplanted in all of these cases. The third condition is the fact that small areas of bone developed along the path by which the transplant was inserted into the testis. The distribution of these areas was such as would be expected if they developed from fragments which rubbed off the forceps when the friable piece of marrow was being inserted. The fourth condition is the finding that in the testis, where there is more opportunity for the main part of the friable graft to spread out, there were always several loci of developing bone, indicating that the sole factor is the distribution of the marrow and not the presence of fragments of endosteum or formed bone.

Levander ('40) does not believe that any of the bone marrow cells persist in a graft. He thinks, instead, that the marrow contains an osteogenic substance which causes osteoblasts to form from the mesenchymal tissue surrounding the graft. These mesenchymal cells he believes migrate into the transplant and are induced to differentiate into osteoblasts which form bone. As further evidence for this thesis he maintains that only with large bone marrow grafts is there sufficient osteogenic substance to induce bone growth and that a 40% alcohol extract of bone produces cartilage and bone when injected into the rectus femoris of the rabbit; the alcohol alone does not do this. In the present experiments the myelogenous and hemopoietic cells die, and all disappear from the graft, but it is thought that the reticulum cells survive and have the capacity to form osteoblasts just as they have been described to do in the normal bone marrow of the pigeon (Bloom, Bloom and McLean, '41). It could well be that the mesenchymal

cells which Levander ('40) saw in the degenerating marrow grafts are really the persisting reticulum cells of the marrow. This would explain the fact that until the present experiments only autotransplants have produced bone. If it were due only to an osteogenic factor dissolved in the marrow, this factor should be effective in homotransplants.

Even if we accept the evidence that the development of bone in marrow transplants is dependent on the presence of an extractable osteogenic substance in the marrow (Levander, '40, '45; Annersten, '41; Bertelsen, '44), it would seem necessary to conclude, from the present experiments, that the marrow reticulum cells are involved. Since the amount of marrow transplanted was too small to contain effective quantities of the osteogenic material, it would have to be produced by some cellular component that not only survived grafting but continued to grow and develop in the new environment. In the absence of the myelogenous and hemopoietic cells, the only such component would be the marrow reticulum cells. The possibility that the reticulum cells might produce an osteogenic substance would agree with the evidence, to be found in the results of all of the marrow grafting experiments reported so far, that it is only when conditions are favorable for grafting that bone develops. It would also explain the observation of Bertelsen ('44) that the marrow contains more osteogenic material than the cortex, epiphysis or periosteum of the bone. The observation that the osteogenic substance is especially concentrated in the lipid fraction of the marrow (Levander and Willistaedt, '46) is also in agreement. McLean ('43) quoted the unpublished work of DeBruyn to the effect that the early work of Levander ('40) and Annersten ('41) had not been repeated. However, the independent finding of an extractable osteogenic factor in bone, cartilage and marrow (Levander, '40; Annersten, '41; Bertelsen, '44; Lacroix, '45a and b) adds evidence that such a factor does exist.

The idea advanced by Levander ('40) that it is only with large marrow grafts that bone develops does not hold in the present experiments, since bone formed in small pieces of

marrow when it was transplanted to the testis and eye, where the resistance to grafting is low. The relatively low incidence of takes even in these regions is probably due primarily to the technical difficulty of transplanting such a friable tissue. In the ear, where larger pieces of marrow could more readily be transplanted, bone failed to develop. Apparently because of the high resistance to grafting in this site the marrow reticulum cells became wandering cells and migrated from the area after hemopoietic elements disappeared. It therefore appears that a grafting site which offers a favorable place for the marrow reticulum cells to become established and to develop is a more essential factor in the formation of bone in marrow grafts than is the amount of marrow transplanted. In such a grafting site it would be possible for the reticulum cells of the mouse to differentiate into osteoblasts and to deposit bone in the same manner as has been demonstrated in the pigeon (Bloom, Bloom and McLean, '41).

The presence of numerous plasma cells, which can arise from the marrow reticulum (Rohr, '36; Gormsen, '42; Bjørneboe, Gormsen and Lundquist, '47), and were seen in the present experiments to transform into fibroblasts, suggests that the plasma cell may be an intermediary in the formation of osteoblasts from the reticulum cells. The regression phase was not as clearly seen. Osteoclasts were observed in a few grafts where some resorption and reorganization of bone was going on, but it is not known whether they were formed from the reticulum cells directly or from osteoblasts and osteocytes. It is also impossible to say from our material whether these osteoclasts can complete the cycle by becoming reticulum cells again.

Since the discovery that estrogen has an effect on bone formation (Zondek, '37; Gardner and Pfeiffer, '38a; Pfeiffer and Gardner, '38), the possibility has occurred to many people that estrogens might be helpful in those cases of fracture where healing is slow or delayed. In the normal animal with experimental fractures, estrogen has had little if any effect. However, estrogen has been used with some success in certain cases

of osteoporosis (Reifenstein and Albright, '47). This would be expected since in the normal animal the factors controlling fracture healing would be operating at a maximum, whereas in the case of osteoporosis, bone formation is slowed down. We do not know how the estrogen produces its effect in the mammal. From the findings in birds the tendency is to assume that it is through a mobilization of salts used in bone formation. We must not forget, however, that in birds this is a physiological condition necessitated by the fact that large amounts of calcium and phosphorus must be available to form the egg shell in a very brief period of time (Charles and Hogben, '33; Gardner and Pfeiffer, '43).

The question arises: does estrogen have any direct effect on bone formation, or does it affect, either directly or indirectly, other factors which hold bone formation in check? The estrogen affects only the endosteal surface of the bone. Therefore, all the new bone formed encroaches on the marrow cavity and for convenience is spoken of as medullary bone. The periosteum, on the other hand, does not seem to be stimulated by estrogen. Since estrogen stimulates the formation of endosteal bone, presumably by inducing the marrow reticulum cells to transform into osteoblasts, it was expected that it would also increase bone formation in transplanted marrow. However, the fact that estrogen had no effect upon the marrow grafts suggests that within the cavity of the bone of the normal animal there are as yet unknown factors which limit the growth of bone from the endosteum and that the estrogen may act to negate these factors and allow endosteal bone to form. It is realized, however, that while this is an attractive hypothesis, the absolute proof is lacking. We also have no evidence how androgen inhibits medullary bone formation following estrogen treatment (Gardner and Pfeiffer, '38b). It does not seem that trauma, pH changes or impaired blood supply influence the formation of bone in these grafts. It is also unlikely that stresses play any important part in the development of bone in the marrow grafts, but it is not denied that they are important in bone organization. All

the evidence so far obtained indicates that the action of estrogen on medullary bone formation is direct. It is effective in hypophysectomized mice, where all the remaining endocrine glands are atrophic (Gardner, '44). The effectiveness of local application has not been adequately tested.

SUMMARY

Bone marrow from the femur was transplanted to the anterior chambers of the eyes of normal female and normal and castrated male mice of the Strong A strain. The normal females, and half each of the normal and castrated males received 16.6 μg of estradiol benzoate weekly. At autopsy after 60 days, organized bone had formed from the marrow transplants in the females and in both intact and castrated males. The grafts did not take as well in the normal as in the castrated males, but the presence of estrogen did not increase the number of takes nor influence the development of bone in the grafts in either case. To eliminate the possibility of stimulation by something in the anterior chamber of the eye, marrow grafts were made into the testis in 20 males and subcutaneously into the ear in another 20 males. In the former site resistance to grafting is very slight, while in the latter it is rather high. Half of the animals in each group received 16.6 μg of estradiol benzoate weekly, and all animals were autopsied after 70 days. The grafted marrow reticulum cells formed bone in the testis and did so equally well whether the host had received estrogen or not. The bone was surrounded by a typical endosteum with numerous osteoblasts and occasional osteoclasts. Immediately outside of this was a layer of loose connective tissue containing numerous plasma cells. The grafting sites in the ears were not found. Presumably the marrow reticulum cells had become tissue wandering cells and had migrated from the area. It is concluded that where resistance to grafting is low marrow reticulum cells form bone. A discussion of what limits medullary bone formation and how it is affected by estrogen is presented.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

The grafts in the eye were stained with Dominici's combination (eosin-orange G and toluidine blue). The grafts in the testis were stained with Ehrlich's hematoxylin and triosin.

1 Bone growing in a marrow transplant in the anterior chamber of the eye. The cornea is at the top of the figure, the lens at the bottom. Note that the bone grows along most of the corneal surface. The host was a castrated male mouse which received no hormone treatment during the 70 days the graft was present. × 100.

2 Bone growing in the anterior chamber of the eye of an intact male mouse after bone marrow had been implanted for 70 days. Cornea at the top of the photograph, lens at the bottom. × 100.

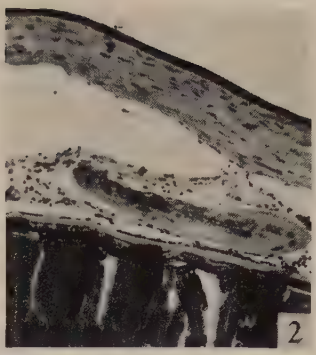
3 The bone which developed in a bone marrow transplant made into the anterior chamber of the eye of a castrated male mouse which had received 16.6 μg of estradiol benzoate weekly during the 10 weeks the graft was present. × 200.

4 The region of the bone marrow transplant in the testis of an otherwise normal mouse. Note the numerous areas of bone growth with damaged seminiferous tubules directly around the graft. The remaining tubules are normal. × 100.

5 Photograph illustrating the development of small fragments of bone along the course by which bone marrow was introduced into the testis. The tunica albuginea was ruptured. A few herniated tubules may be seen at the top right of the picture. A portion of the tunica albuginea may be seen extending somewhat diagonaly from the rupture to the surface of the testis at the left of the photograph. Note the distribution of the small areas of bone growth from this rupture into the region of the testis where the main portion of the bone marrow was placed. This animal had received 16.6 μg of estradiol benzoate weekly for the 10 weeks the graft was present. × 100.

6 The area of the bone marrow graft in the testis of an otherwise normal male mouse showing good development of bone with minimal disturbance to the seminal tubules. Numerous plasma cells and collagenous connective tissue fibers are present around the bone fragments. Transition stages between plasma cells and fibroblasts can be found in these areas. × 100.

1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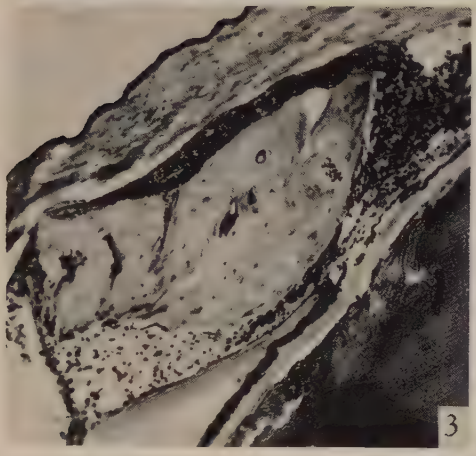
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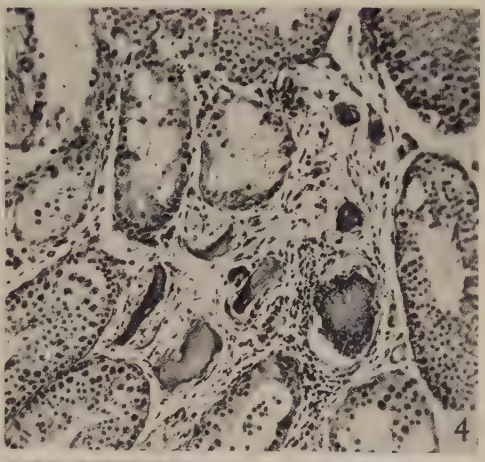
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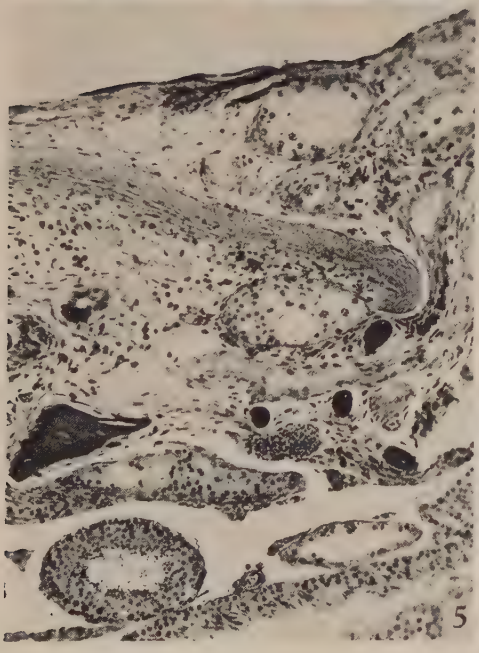
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SIGNIFANCE OF PLACENTAL AND BIRTH-WEIGHT RATIOS

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FOUR FIGURES

The human fetus during the whole of gestation is so completely dependent upon placental transfer of all metabolites that some measurable correlation of placental structure or weight with birthweight is predicated. Data have been accumulated since 1939 to test the hypothesis.

PRESENT STATUS

Adair and Theelander summarized the scant literature on this subject in 1925. Most observers have noted that very large babies had large placentas but otherwise saw no correlation. Adair and Theelander ('25) studied 392 placentas, separating out 18 premature cases. The average weight was 473 gm and the ratio for the group was 1/7.27 with a correlation coefficient of 0.63. Harer ('36) studied 1000 placentas anatomically but his paper does not utilize weight data. Dow and Torpin ('39) studied 269 birth and placental weights, superficial placental areas and gestational sac areas and drew curves of correlation between the 4 variables. The methods used in their work, because of the small sample, differ so much from the data we present that comparison is not possible. Wolfram ('41) was not available until the present paper was completed but it offers a different approach to similar data. The data for each of 1984 births were distributed according to a gestation age calculated from the known

menstrual cycle by the method of Naegele with some modifications. The final method itself is not described nor is there direct reference to it. However, it is stated to be successful to within an average of 7 days. All birth data from cases with very unusual or irregular periods were discarded. Mean birthweights (3250 gm) and placental weights (429 gm) are slightly lower than in our figures but the form of the distribution looks very similar. Correlations are pictured but not calculated and appear to show the same wide variations within each class that has been seen by others. Prematures calculated by his method cover a range in weight from 1000 up to 3700 gm and mean values for postmatures also range from 2700 to 4300 gm. Considering mean values there is a positive and regular correlation of birthweight and placental weight showing a decreasing ratio with increased weight.

MATERIAL AND METHODS

We studied 1517 placentas and birth records from normal single live births in Galveston, mostly in 1939-1941. We excluded stillbirths because they constitute a separate problem with a different series of placental-fetal weight ratios and a number of artifacts. Caesarian sections were omitted because the placentas are invariably engorged with blood and the exact time relationships to normal births are not available on the charts. We could see no a priori reason for separation of the sexes nor for distinctions of color. We found it impractical to omit the premature infants because prematurity is defined by clinicians on practical grounds to include all small babies that need extra care even though it also includes many small fully formed babies not in need of such care. Multiple births, generally premature, form a separate study group.

Some variables are difficult to control or evaluate. The placentas contained variable proportions of maternal blood but except for removal of all clots external to the decidua we did not attempt to remove more by compression. The amount that may be expressed is surprisingly small though all pla-

centas which were removed manually show blanching. If through impatience the cord is tied prematurely more fetal blood is retained. According to Harer ('36) the decidua is 10 mm thick at 4 months gestation and decreases to 1–2 mm at term. It cannot be removed. Infarcts are so irregular and account for so little weight that they must be left in situ.

We attempted to prepare placentas uniformly for weighing. The nonplacental chorion and amnion weigh 40% as much as the placenta among birthweights below 2500 gm, decreasing to 29% for the smaller full term babies and 21% among the heaviest placentas and babies. This conclusion was drawn from 250 weighings. Individual cases showed extreme variation from 5% to 85% independent of placental weight. The cord also is an independent variable averaging 10% with a range rate from 3% to 27% of placental weight. We, therefore, trimmed the membranes close to the placental margin and removed both amnion and cord. Most placentas were studied fresh or chilled, though a series of 200 were in formalin. No significant difference was noted because of fixation. Three people carried out most of the placental weighing.

Birth records were abstracted within 24 hours and any apparent discrepancies were investigated and corrected. Some errors are bound to enter a large series where many people are concerned with weighing, recording, and converting weights from pounds to grams. The work was done in two hospitals.

STUDY OF THE DATA

In a preliminary paper, Sinclair ('48) plotted the distribution of birthweights for 1457 births according to birthweight classes. All live births were included. There was no bimodality because of prematurity but asymmetry due to its presence was evident. Placental weights were then plotted for the same birthweight classes. There is a definite linear relation between placental weight and birthweight with rather wide variation for each class. The trend was reduced to two formulae.

The ratios of placental to fetal weights for these 1457 births are shown in figure 1 B. In the region of full term births the ratios fall with increased birthweight from 0.158 to 0.129. Premature ratios are high, increasing with decreased birthweight. The extreme low ratios for each class are plotted separately in figure 1 A. The value 0.064 is the lowest ratio for several classes. One placenta with a ratio of 0.054 was compressed and bloodless. Post-mature ratios are high.

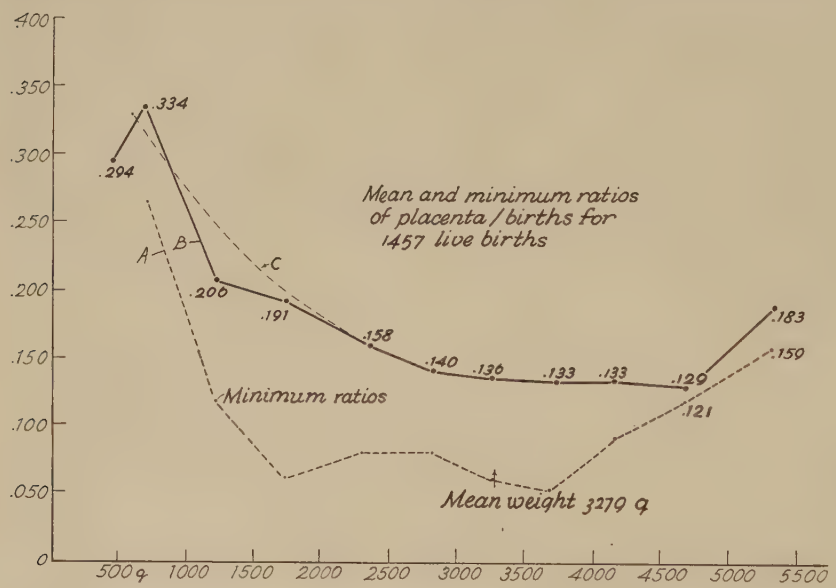

Fig. 1 Mean and minimum placental-birthweight ratios distributed according to birth weight classes for 1457 live births. A. Graph of the minimum ratios recorded for each class. B. Mean values of ratios recorded for each weight class. C. A theoretical correction for intrauterine growth curve for those that are not born prematurely.

In figure 2 the birthweights for 1517 single live births are distributed according to placental weight classes differing by 50 gm. Curve A for the total group shows the number in each and the mean value of each class. The curve is smooth for the main distribution and inflects near the mean value, 3274 gm. It deviates downward for small placentas and upward for large placentas. In order to visualize the distribution

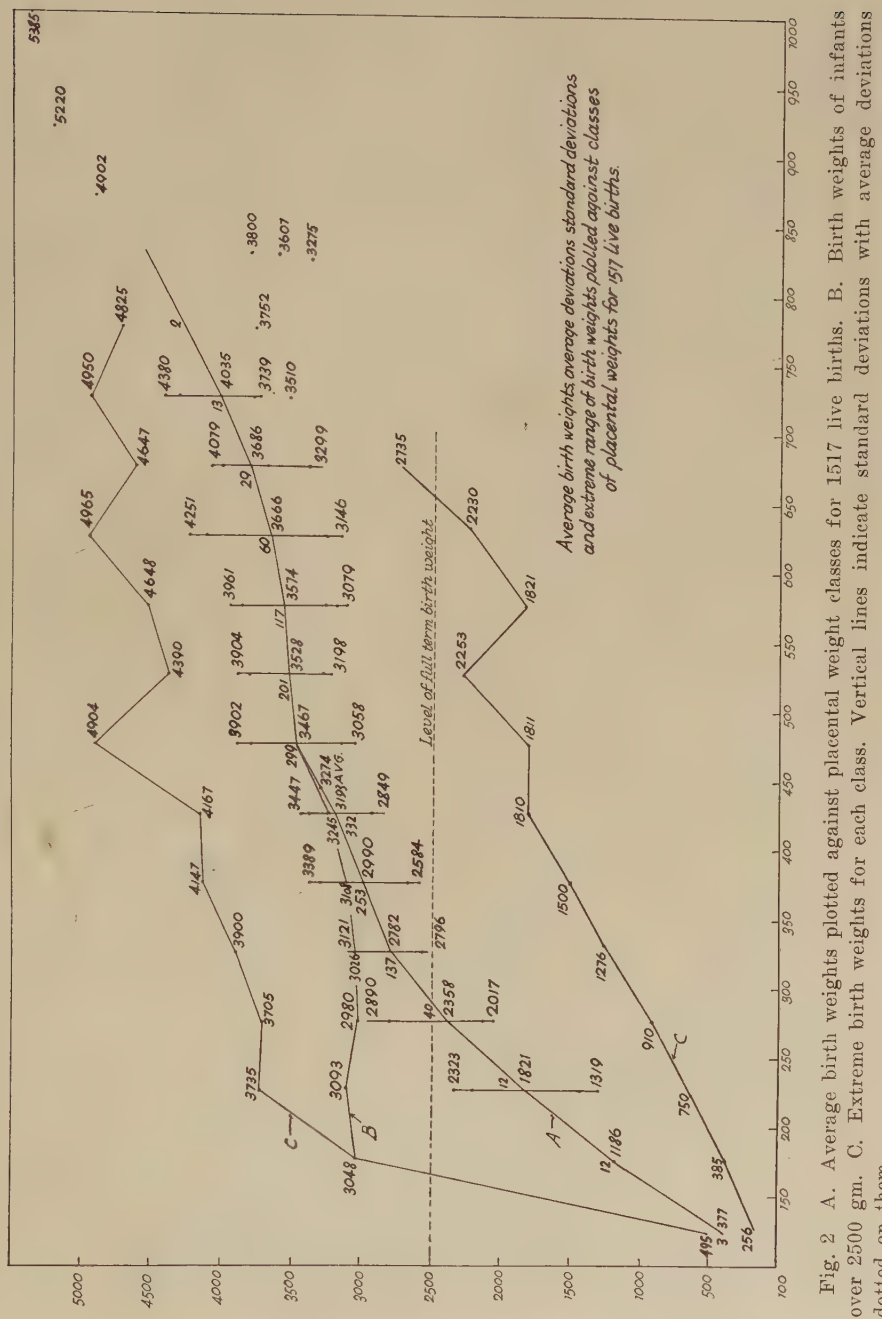

Fig. 2 A. Average birth weights plotted against placental weight classes for 1517 live births. B. Birth weights of infants over 2500 gm. C. Extreme birth weights for each class. Vertical lines indicate standard deviations with average deviations dotted on them.

better, vertical lines are drawn indicating the standard deviations from the average of each class. The outline of total range is also drawn, using extreme values. It is clear that there is a definite trend but with wide variation for each class. If we accept the clinical definition of prematurity and draw a line at 2500 gm subtracting the prematures from the population, curve B shows that the average birthweight rises with placental weight, but it also shows that many of these small placentas in general belong to very heavy infants but this is not invariable.

In figure 3, graph A, the 1517 placentas are distributed according to placental weight classes. The average placental weight is 444 gm. In graph B the infants of less than 2500 gm are subtracted from the population as we did in figure 2. These placentas of supposedly premature infants constitute 93% of the 175 gm class and are reduced for succeeding classes to 67%, 30%, 25%, 12.6%, 5%, 2.3%, 1%. Some small infants have placentas in the 625 gm class. In graph C the ratios of placentas to birthweights are plotted for the total group. Ratios are found to be very high for small placentas but they fall to a minimum of 0.112 at almost the lowest level of the main distribution and rise continuously to 0.182 at the other side of the same distribution. Curve D for premature ratios (2500 gm or less) shows a similar general form but the whole is at a much higher level. After separating the premature group from the total, the resulting full term ratios are plotted in curve E. Here the ratios of infants above 2500 gm are seen to rise as a straight line from 0.064 to 0.182 or higher. In other words, the ratio of placental to birthweight increases linearly with placental weight.

The placental-birthweight ratio was found to decrease linearly with birthweight. This leaves an apparent paradox. It is possible to calculate a series of birthweights corresponding to given placental weights from either curve. When this is done, it is found that the two series do not lie in the same axis. Upon a base of coordinates for birthweight from left to right and placental weight from front to back the two series

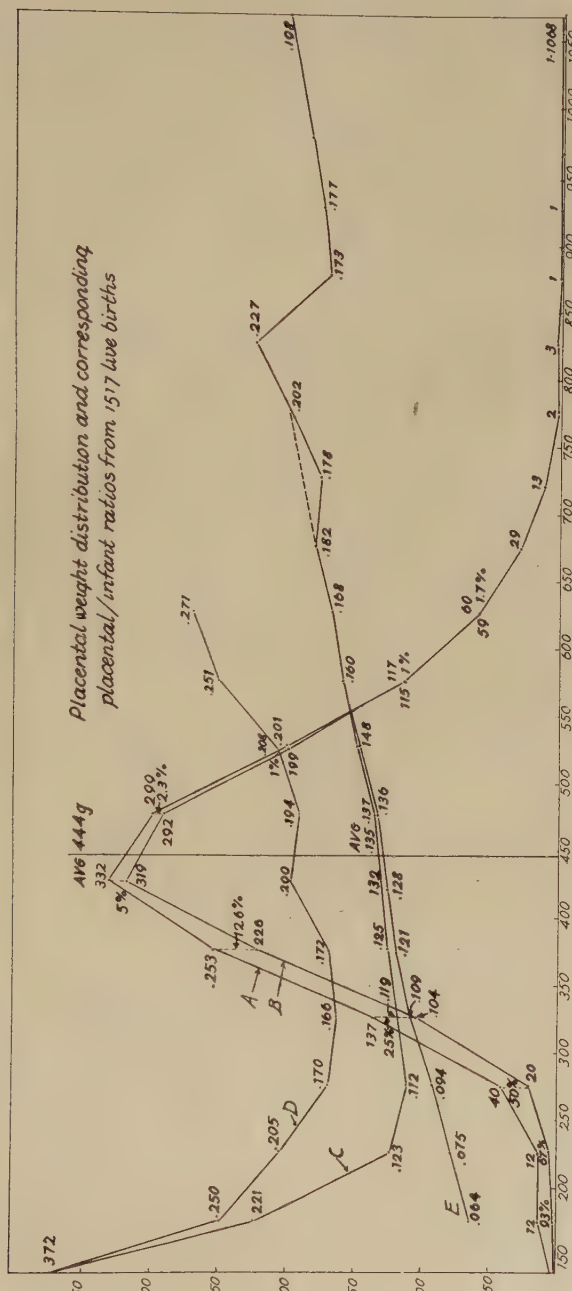

Fig. 3 A. Placental distributions by weight classes for 1517 live births. B. Distribution of placentas from infants of less than 2500 gm birth weight subtracted from the total distribution. C. Placental-weight birthweight ratios for the whole population. D. Placental-weight birthweight ratios for the premature group. E. Placental-weight birthweight ratios for full term infants.

are plotted in figure 4. Then from each point at which a value is established a vertical line equal to the ratio is raised. This then gives two ratio curves which cross one another at the average value for placenta and birthweight and which show the characteristic rise in one and decline in the other. The stereogram, figure 4, visualizes the relationship of the two and resolves the paradox.

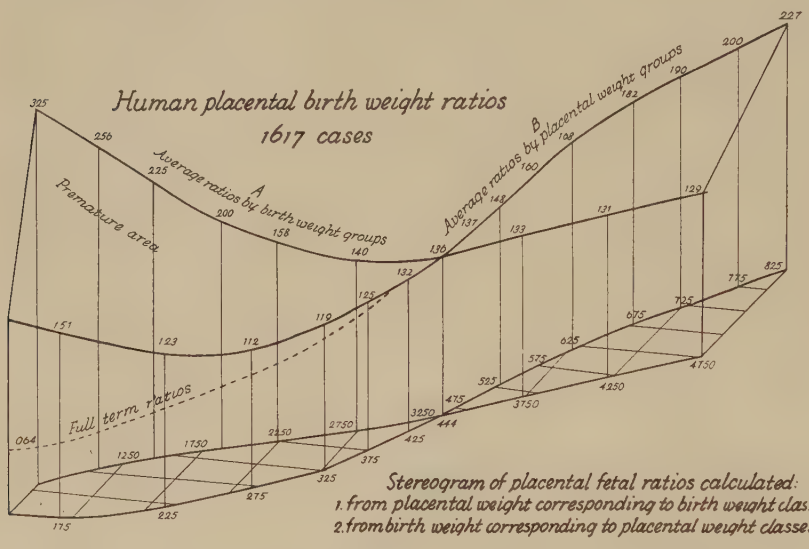
Human placental birth weight ratios
1617 cases
Premature area
Average ratios by birth weight groups
A
Average ratios by placental weight groups
B
Full term ratios
Stereogram of placental fetal ratios calculated:
1. from placental weight corresponding to birth weight classes
2. from birth weight corresponding to placental weight classes

Fig. 4 Sterogram of placental fetal birthweight ratios calculated from: A. The distribution of birthweight groups. B. The distribution of placental weight groups. On the latter the full term ratios are superimposed.

DISCUSSION

The present study of placental-birthweight ratios differs from previous work in demonstrating linear relationships for the mean values of classes whether we use birthweight or placental weight as the basis of such classes. In either method of plotting the data there is an increase of placental weight with increased birthweight. This much is in line with physiological expectation. The trends by the two methods differ. If birth represents an approach to the limits of reserve capacity of the placenta one would expect a much closer corre-

lation and less variation. There seems to be two explanations possible. Either the weight of the placenta does not give the proper clue to its physiological state or the placenta plays no part in the onset of parturition.

Abortion occurs from many causes, one of the most important being the death of the fetus. This may be initiated by infection or anoxia through premature separation of the placenta but as far as can be seen placental structure has little to do with it. Many early abortuses are congenitally abnormal and most of the others show petechiae or gross hemorrhage. Serological reactions are under suspicion as a common factor. Brooksby and Newton ('38) indicated a poorly analyzed endocrine function for the placenta related to water metabolism. Other functions have been established but none of them are known to reach a critical value at birth. The effects of distention must be taken into account. It is well known that multiple births are almost invariably premature in man. Kirsch ('38), Brooksby and Newton ('38), and Reynolds and Foster ('39) studying other mammals concluded that the length of gestation varied with the total uterine contents, being considerably shortened by large litters. Activity also plays a part. The most important factor according to Snyder ('38) is the gradual strengthening of the muscle tone and other phenomena of the menstrual cycle of which birth is regarded as a resumption. Loewenhardt, as far back as 1872, had postulated that the gestation period equalled 10 menstrual cycles characteristic of a particular woman. We would add to this the cyclic contraction of placental vessels seen by Schmitt ('29) which, taken together with the periodic contraction of uterine vessels which Markee ('40) describes as initiating menstruation, could, if simultaneous, produce a crisis of anoxemia. Such an end point then would be no more exactly related to a fixed gestation period than the length of the menstrual period itself is fixed. The method used by Wolfram ('41) calculated the gestation period from the individual's menstrual cycle, the calculated period being shortened for a short cycle and lengthened for a long one. The

ability of the placenta to contract was shown by DeBreuil and Riviere ('32) who demonstrated large quantities of very fine smooth muscle cells outside of blood vessels. Arey ('48) has presented a method of demonstrating these fibers clearly. The rapidity of growth just preceding birth which Potter and Adair ('40) indicate at about 600 gm per week must logically bring about an oxygen crisis unless the placenta has an enormous reserve capacity for transfer.

Does either placental weight or placental-birthweight ratio express the true state of the placenta with regard to its reserve capacity to permit further growth? It is known that the placenta at first grows rapidly and is many times the weight of the embryo. Equivalence is reached about the 4th month, after which the fetus increased its weight 30–35 times but the placenta only 4.5–5 times. It seems evident that the placenta permits this tremendous growth of the fetus by increasing its own capacity to transfer metabolites. Many studies of the structure of villi during the course of gestation have shown a picture of aging during which the epithelium is reduced, fibrous tissue increased, the average diameter of villi is reduced and vessels become prominent.

Microscopic study of the placenta

Applying this concept to our problem we made sections of 750 placentas. We separated our material into three groups: those with ratios above 0.2, those with ratios below 0.12, and the larger number between these figures. An examination of these groups indicated clearly that there is a correspondence between ratio and microscopic structure. Those with high ratios irrespective of the placental weight were relatively immature, with large villi, intact epithelium, very cellular villi and small blood vessels. Those with low ratios in all weight classes showed relative maturity with small villi whose epithelium was reduced to nuclear knots, the villi lacked young fibroblasts and the vessels were relatively large and blood filled.

Significance of microscopic structure

If it is generally true that high ratio placentas are structurally immature it means that they are physiologically equal in efficiency to considerably smaller low ratio placentas. The wide variation seen in the ratios of any one class of placentas on the basis of either placental or birthweights is much narrower than the weights indicate. If this microscopic structure could be reduced to a coefficient of efficiency the correlation of placental and birthweight would be much higher than it appears. There is still the problem of why there is so much variation in placental structure at birth. There seem to be two possible explanations. It is recognized that physiological and chronological age are quite independent during development after birth and that this independence is based on genetic patterns. There is reason to believe that the placenta is no more exempt from the action of genes than is the fetus. At the same incubation age chicks of different varieties differ tremendously in size. With any method of calculating the length of gestation period great variation in placental and birthweights are found. The second explanation is that the length of gestation itself is quite variable and the degree of maturity of the placenta varies in general with it. Potter and Adair ('40) present these variations in length of gestation in tabular form and indicate that variations of a week or two are not uncommon for full term babies. Wolfram ('41) based his distribution on calculated gestation periods varying 28 days over or under the mean date. Most obstetricians feel that these calculations are inexact. Our data show that there is considerable overlap between late premature and early mature birth and placental weights.

Related variables

Are there other variables beside length of gestation and genetic patterns of growth which enter our problem? Mitchell-Nelson ('45) states that first born males weight 137 gm and later males weigh 92 gm more than females at birth. Our data

correspond but we find that the placental-birthweight ratios correspond to average weights and not sex. Colored infants also are a little smaller than white and their ratios correspond to weight. The site of implantation is a significant variable. It is indicated by the position of the cord on the placenta. A marginal cord always means malposition and a vascular handicap. We studied 121 placentas with marginal cords. The babies weighed 3195 gm or 84 gm below normal single live births. The mean ratio is 0.130 for infants of mean weight. For 3195 gm weight it should be 0.135 but it is actually found to be 0.147. It takes more placenta to carry the load under a disadvantage of position.

Other factors have been published. Nutritional state is cited. Sinclair ('48) finds it of little consequence except in extreme cases. The actual weight of parents is important as indicating a variation in genes. Homogeneous as compared to heterogeneous parental blood groups are said to produce larger children. First borns weigh slightly less than later children.

The high ratios obtained from babies well above the normal distribution of birthweights pose a problem. When viewed in figure 3 they form a continuation of the rising line of ratios for the normal population but when viewed in figure 1 they are discontinuous. There are not many of them and they are frequently related to pathological conditions. Thus the hydrops infant of Rh incompatibility falls here. Some are reactors to maternal diabetes, to hypothyroidism, etc. Most of them are lethargic at birth. We did not suspect this discontinuity at the time of birth and with the exception of two cases of Rh erythroblastosis fetalis did not determine what the pathology was.

CONCLUSIONS

The human placenta undergoes microscopic changes during gestation which increase its capacity to transfer metabolites and, therefore, compensate for the fact that it grows much slower than the fetus. At birth placentas still grow a con-

siderable range of microscopic structure. This structure in different placentas corresponds to the placental weight-birthweight ratio. Relative immaturity in the placenta corresponds to a high ratio and relative maturity to a low ratio. The lowest ratio reached is 0.064 for most classes of full term placentas. Both placental weight and ratio increase with birthweight when distributed by placental group classes, the increase being linear for all full term births. Prematurity and postmaturity are both characterized by high ratios. Malposition, indicated by a marginal cord, also corresponds to a smaller than normal baby and a higher placental ratio.

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CORPORA AMYLACEA IN THE PROSTATIC SECRETION AND EXPERIMENTS ON THE FORMATION OF A COPULATORY PLUG IN SOME INSECTIVORES

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FIVE FIGURES

INTRODUCTION

The occurrence of a copulatory plug in the vagina after mating has been recorded in representatives of 4 orders of mammals: Marsupialia, Insectivora, Chiroptera, and Rodentia. Engle ('26a) summarized the known occurrences to that date. The phenomenon is best understood and most widely recorded in the Rodentia for which Stockard and Papanicolaou ('19) and more recently Moore and Gallagher ('30) have provided an historical summary of available information. Walker ('10) showed that, in the rat, the vaginal plug is formed from the secretion of the seminal vesicles coagulated by an ingredient from the anterior lobes of the prostate gland and this physiological mechanism was reaffirmed by Engle ('26b) in the guinea pig. Among the marsupials, Hartman ('24) found a copulatory plug in the opossum but believes it to be formed differently from that of rodents, observing that the plug is formed from the fluid of the lateral vaginal canals coagulated by the seminal fluid.

In the Chiroptera a vaginal plug has been recorded in the Rhinolophidae and Vespertilionidae although apparently of quite different character in the two families. Rollinat and Trouessart (1897) state that in the horse-shoe bats the plug

is formed from the secretions of the male "urethral" glands, while in the Vespertilionidae the plug is known to be formed from the cornified epithelium of the upper vagina itself which sloughs off into the lumen after copulation (Grosser, '03 and Courrier, '25). Wimsatt ('44) did not find a vaginal plug as a constant feature in hibernating bats of the Myotis lucifugus and Eptesicus fuscus species. When found, it consisted almost entirely of cornified epithelial cells.

Camus and Gley (1899) recorded the presence of a copulatory plug in an insectivore for the first time when they reported on its occurrence and mode of formation in the European hedge-hog (Erinaceus). Because of the confused state of the nomenclature of the accessory reproductive glands in this animal both then and now, it is not possible to classify with certainty the glands involved in the formation of the plug. In Erinaceus there are three prominent pairs of accessory glands, one pair lying dorsal to the bladder, a second pair somewhat ventral, and a third pair lying far caudad outside the pelvis and body cavity. The first pair have been identified by different authors as seminal vesicles or as lobes of the prostate called "Prostate I." The second pair have been consistently referred to as prostates ("internal prostate" or "prostate II") while the third pair have been regarded variously as Cowper's glands, lobes of the prostate ("external prostate" or "Prostate III") or glands *sui generis* in Erinaceus (Rauther, '04; Disselhorst, '04; Linton, '07; Kaudern, '10; Courrier, '27; and Allanson, '34). An additional pair of glands embedded in the muscle of the bulbus penis were identified by Marshall ('11) as Cowper's glands in Erinaceus. According to the above mentioned work of Camus and Gley the vaginal plug is formed from the secretion of the "seminal vesicles" (Prostate I of authors) coagulated by some ingredient in the secretion of Cowper's glands (Prostate III of authors). They found that the coagulation formed a thick paste, not a waxy material as in rodents, and that the reaction was a specific one.

The occurrence of a vaginal copulation plug in the European mole (Talpa) has been recorded by Courrier ('27) who further states that "the prostatic liquid has the property to produce agglutination of the spermatozoa previously taken from the testicle or epididymis and we have seen that the product of Cowper's gland coagulates the prostatic liquid." Mathews ('35), working on females of Talpa, stated that "The presence of a definite mucous plug in the uterovaginal canal after coitus has not been observed." He later states, however, that in many cases during early pregnancy the lower part of the uterovaginal canal contains a mucoid substance which appears to be the secretion of the accessory glands of both sexes mixed with desquamated epithelial cells of the uterovaginal canal. An additional record of the occurrence of a copulatory plug in insectivores was made by Eadie ('39) for the American mole, Parascalops, where a firm, pasty mass fills the lower part of the uterovaginal canal in recently mated females.

In the course of a study on the homologies of the accessory reproductive glands in American insectivores, it was discovered that the principal and normal product of the prostate gland in the star-nosed mole (Condylura) is a mass of corpora amylacea resembling the so-called prostatic concretions of the human prostate (Eadie, '48). In an attempt to determine the possible function of these bodies and the mode of formation of the copulatory plug in American moles, a number of experiments were initiated.

MATERIALS AND METHODS

The difficulty of trapping live moles and maintaining them alive in the laboratory for any length of time precluded this approach to the problem. Therefore as many moles (Condylura) as possible were collected in killing traps and brought to the laboratory for examination and experimentation. By visiting traps frequently it was possible to obtain freshly killed moles, some still warm. For the purposes desired, it was necessary to collect these moles during the rather restricted mating period of late March and April. Twenty-six

specimens were collected of which 14 were males and 12 females. In addition, 2 males of the genus Parascalops were collected. The females were dissected, examined for the presence of a copulatory plug, and the condition of the reproductive tract noted. The males were dissected and the accessory glands excised for experimental use.

OBSERVATIONS

The copulatory plug. The fluid from the excised prostate of a freshly killed male Condylura is viscous and milky white. The viscosity increases rapidly as the material stands and with time after death. In animals dead over one hour the product when expressed from the gland is a pasty mass. The fresh secretion from Cowper's gland is a thick, pure white fluid which also thickens quickly on standing. To observe the possible mechanics of coagulation, a number of tests were made using the products of the excised glands of freshly killed males. For the first test the expressed fluid of the prostate was divided into three equal portions on a glass slide. One portion was left as a control; to the second was added fluid and sperm from the cauda epididymis; and to the third was added a droplet from Cowper's gland. This latter mixture on stirring with a needle showed immediate coagulation as evidenced by the formation of definite clots. The other two portions remained fluid and unclotted upon stirring. This test was repeated with three other males with the same results.

To check these results, two additional tests were devised. The secretion from the prostate was divided into two equal portions on a glass slide. One was left as a control and to the other was added a drop of Cowper's secretion. Both portions were stirred vigorously with a needle and a cover slip dropped gently upon each. The aliquot used as a control spread evenly and quickly under the weight of the cover slip while the weight of a cover slip was inadequate to spread the mixture of the prostatic product plus Cowper's secretion. This was taken as corroborative evidence of a coagulation in this latter portion. The third test involved the use of viable sperm from the cauda

epididymis. Two equal portions of the prostatic product were placed on a slide and one was mixed with a droplet from Cowper's gland. Then to both was added a drop of fluid containing sperm from the cauda epididymis. Upon examination under the microscope it was at once apparent that the sperm contained in the prostate-Cowper's gland mixture exhibited a greatly restricted activity when compared to the control. This was considered as evidence of the coagulation that had occurred. Each of these latter tests were repeated with the same results on two additional specimens. The same tests were repeated on two Parascalops males with similar results.

It is therefore concluded that in American moles of the genera Condylura and Parascalops the prostatic product is coagulated by an ingredient of Cowper's gland to form a copulatory mass. This has been found in the vagino-uterine canal of recently mated Parascalops specimens. Attempts to capture a female specimen of Condylura which had but recently mated and possessed a copulation plug in the vagina were unsuccessful. All female specimens captured either showed visible uterine swellings of pregnancy or had not yet mated as shown by examination of the genital tract. In a larger series from the mating period it is expected that some will be found with the vaginal plug as in Parascalops. In this latter genus it is not known how long the copulatory plug persists in the females after mating but those specimens showing incipient embryonic swellings in the uterine cornua had lost all trace of it. This was also true in female specimens of Condylura collected at a similar stage.

The product of the prostate gland in some insectivores. Disselhorst ('04) observed what he described as large egg-shaped bodies in the prostate secretion of Talpa. He was uncertain whether these were parasites or amorphous protoplasmic bodies. Grosz ('05) observed that the product of the prostate gland was granular and saw in it the "egg-shaped bodies" described by Disselhorst. "Numerous floating particles resembling irregularly shaped crystals" were observed

by Marshall ('11) in the fluid from the so-called seminal vesicles (Prostate I) of Erinaceus. Hopkins ('11) made a chemical analysis of these bodies at the request of Marshall and described them as "transparent somewhat irregular masses" consisting chemically of a protein containing phosphorus. He further described their resistance to the action of a variety of chemical reagents and estimated that they formed a large proportion of the total proteins of the "vesicular fluid." Courrier ('27) observed that the "seminal vesicle" of Erinaceus contains sympexia (concretions) and stated that the lumina of the prostate gland of Talpa are filled with a liquid in which float "voluminous concretions."

When these peculiar bodies were found to constitute the chief product of the prostate gland in Condylura (fig. 1) they were identified as corpora amylacea (Eadie, '48). Examination of two male specimens of Brewer's mole (Parascalops) in breeding condition shows that the prostatic fluid consists chiefly of a mass of suspended amyloid bodies in this genus also (fig. 2). It is thus apparent that the occurrence of corpora amylacea as the principal product of the prostate gland is probably widespread among the Talpidae since it has been recorded in representatives of three subfamilies, Talpinae, Scalopinae, and Condylurinae. The occurrence of a similar gland product in Erinaceus extends the record to another family of Insectivora, Erinaceidae, and suggests that the phenomenon may be more widespread among insectivores than we know at present. I have found amyloid bodies in the prostate glands of the soricid genera Sorex (fig. 5) and Blarina (fig. 4) but in no case did they constitute a significant portion of the gland product, occurring only occasionally and sporadically as in the human prostate. It is significant that available evidence indicates that a copulatory plug does not occur in the Soricidae (Pearson, '44).

The problem of the possible special function of the amyloid bodies composing the bulk of the copulatory mass in insectivores is an intriguing one. Their presence certainly aids in determining the final consistency of the copulatory mass and

their principle function may be that of giving "body" to the mass not sufficiently provided by the coagulatory process alone. Observing an experimentally prepared fresh copulatory mass under the microscope leads one to infer that these bodies may serve also as an aid to the agglutination and temporary immobilization of the sperm cells since these cells are of necessity crowded into the rather scanty fluid in the interstices between the amyloid bodies where coagulation occurs. Adsorptive phenomena associated with the great amount of surface area provided by these bodies may permit them to act as a physical aid in the transportation of the sperm cells. This latter function is suggested by the appearance of fresh and fixed smears of a mixture of the copulatory mass ingredients prepared in the laboratory (fig. 3). There is a definite concentration of spermatozoa at and near the surfaces of the amyloid bodies.

DISCUSSION

It is generally considered that the copulatory plug of rodents functions to close the os uteri in order to prevent the loss of spermatozoa from the uterus thus increasing the degree of mating success. A similar function is ascribed to the plug in the opossum by Hartman ('24). In moles there is no os uteri as revealed by examination of the female reproductive tract in Condylura and Parascalops and by the work of Mathews ('35) on Talpa. The uterine cornua open into a median uterovaginal canal. With this anatomical condition, the presence of a copulatory plug after mating would seem to have a distinct advantage in assuring the retention of the seminal mass and consequent mating success.

It has been shown that the prostate glands of talpids and soricids are homologous on the basis of ontogenetic evidence (Eadie, '47a, '47b). The physiological ability of this gland in the two families to produce a similar specific product, corpora amylacea, would seem to be additional evidence of their homology, even though the production of these bodies is quite limited in the soricids. An extension of this reasoning leads

one to assume that the so-called "seminal vesicles" of Erinaceus, which also apparently produce corpora amylacea, will, as some authors have maintained, eventually prove to be lobes of the prostate gland when ontogenetic evidence becomes available. The fact that the known Cowper's glands of talpids produce the coagulating ingredient of the copulatory mass leads one also to the possibility that the similarly located glands of Erinaceus (variously identified), which perform the same function, will prove to be homologous with the Cowper's glands of other insectivores.

The production of corpora amylacea by the prostate glands of certain insectivores repeats a physiological phenomenon of the prostate gland of man and some other mammals. These "prostatic concretions" of man have been often regarded as abnormal constituents although Moore ('36) regards the formation of corpora amylacea as a normal function of the human prostate. The production of these bodies as *the normal and chief product* of the prostate gland in talpids and erinaceids should have some significance to students of human prostate physiology. Also, in view of the contention that Primates have evolved from some early type of insectivore such as a protoerinaceoid group (Simpson, '45), these facts may be of some significance to taxonomists. It would be interesting to know the extent and degree of occurrence of these amyloid bodies in the prostate glands of other Primates than man and in other groups of insectivores.

SUMMARY

Data on the occurrence of a copulatory plug in the Insectivora is reviewed and the phenomenon investigated in the genera Condylura and Parascalops. In these two genera the product of the prostate gland is coagulated by the secretion from Cowper's gland to form a thick, pasty mass which fills the lower half of the uterovaginal canal in the recently mated female mole. This copulatory mass was successfully produced in the laboratory by using fluid from the excised glands of freshly trapped moles.

The production of corpora amylacea as the chief and normal product of the prostate gland in certain insectivores is considered. It is suggested that these bodies may function to give consistency to the copulatory mass, to aid in agglutination and temporary immobilization of the sperm cells, and to aid in the physical transportation of the sperm cells through adsorptive surface phenomena.

The significance of a copulatory plug in insectivores is discussed in relation to the homologies of the male accessory glands and in relation to absence of the os uteri in moles. It is suggested that the normal production of corpora amylacea in insectivores may have some significance for human prostate physiology and primate evolution.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

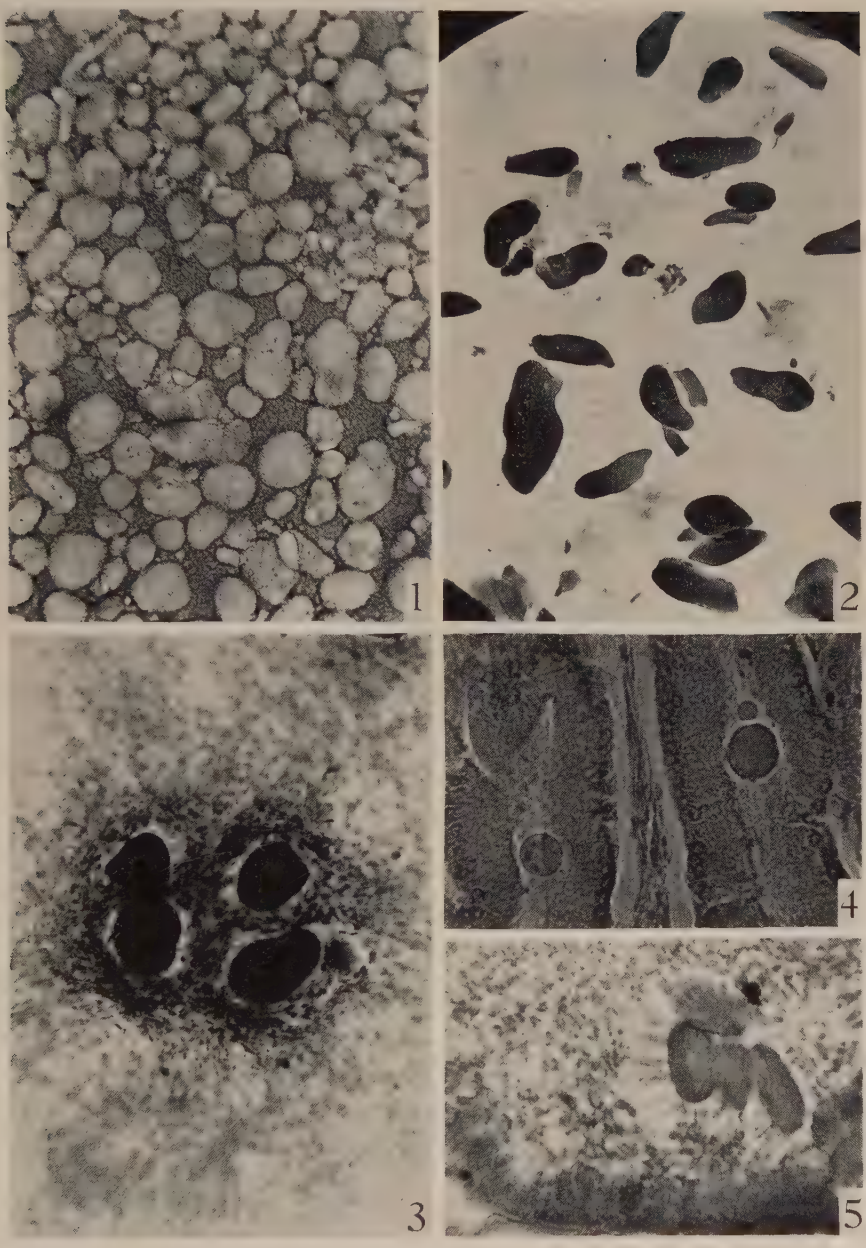
1 Photograph of a dried smear preparation of the prostatic product from Condylura to show range in size and shape of corpora amylacea. Their granular appearance is due to the dried remains of the prostatic fluid adhering to their normally smooth surfaces. Unstained. × 100.

2 A dried and stained smear preparation of the corpora amylacea from the prostate gland of Parascalops. Note that they are more irregular in form than those of Condylura. × 150.

3 View of isolated corpora amylacea in the stained smear of a freshly prepared copulatory mass from Condylura to show the heavy concentration of sperm cells at and near the surface of the amyloid bodies. × 300.

4 Section through the prostate gland of breeding male Blarina to show presence of occasional corpora amylacea in the alveoli. × 600.

5 A thick section through the prostate gland of a breeding male Sorex to show a group of 4 corpora amylacea in the lumen of an alveolus. These are of scattered occurrence only. × 350.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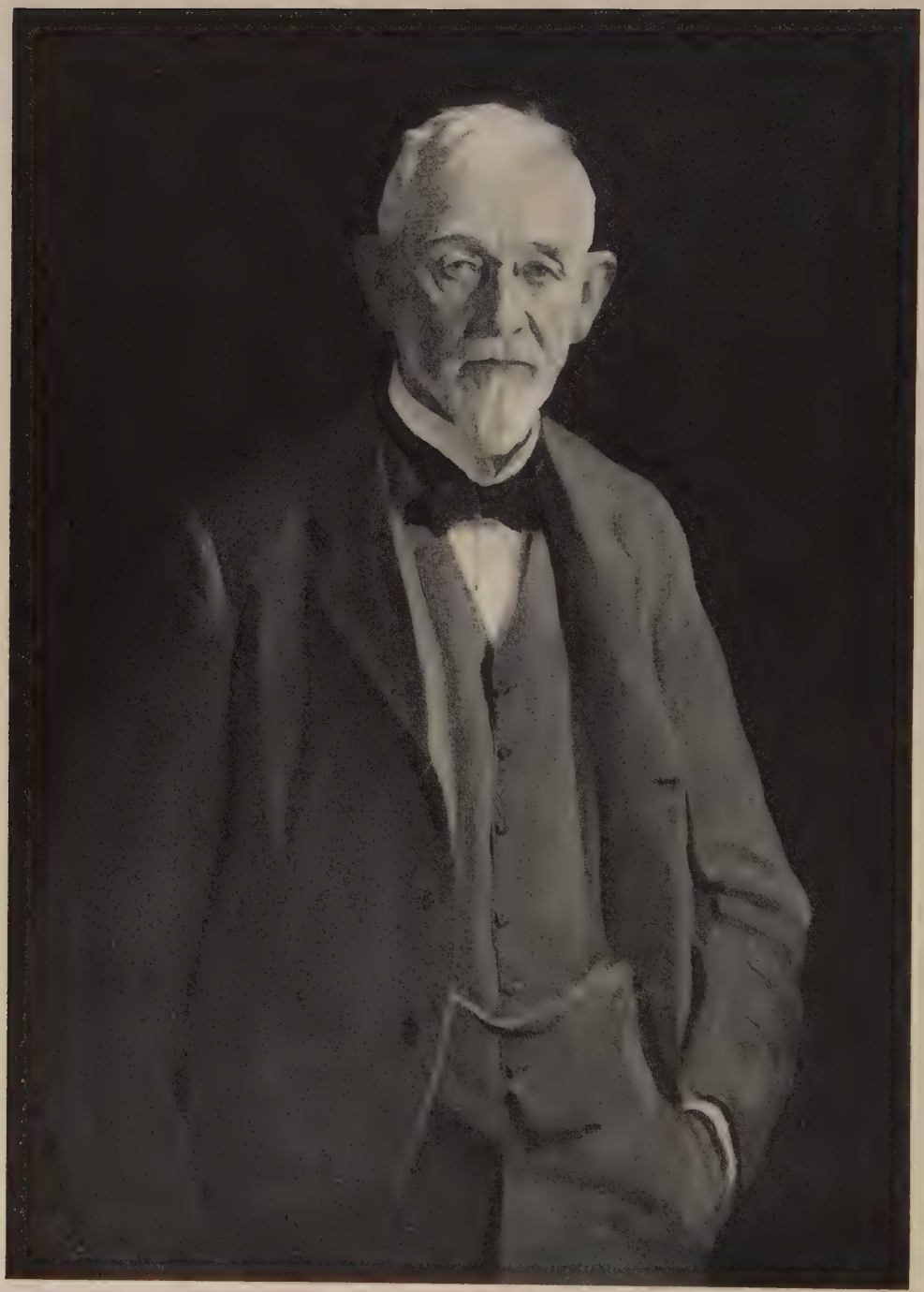
EDWARD LAURENS MARK

1847–1946



E. L. Mark

EDWARD LAURENS MARK

(May 30, 1847 – December 16, 1946)

On December 16, 1946 Dr. Edward Laurens Mark, Hersey Professor of Anatomy at Harvard University for 36 years, died in his hundredth year. Dr. Mark retained a keen and active interest in biological problems almost to the end. At the New York meeting of the Anatomists in 1942 he astounded his fellow anatomists in showing the same inquiring mind and critical analysis of problems which had characterized his entire professional life. Chatting and laughing he enjoyed the Anatomists' smoker, being one of the last to turn in.

Dr. Mark graduated from the University of Michigan in 1871. He was instructor in mathematics at Michigan and on the U. S. Northwest boundary survey for the next two years. In 1873 he went to study with Leuckart, and in 1876 received his Ph.D. degree from the University of Leipzig. This was the period which marked the birth of modern cytology and things were happening rapidly on the continent. We need only think of the discoveries of the Hertwigs, Strasburger, Flemming, Edward van Beneden, Fol, Girard, Bütschli and many others to see the environment in which young Mark found himself. After a period of study at Trieste, he came to Harvard as instructor in zoölogy in 1877. He immediately started work on the cytology of Limax and in 4 years completed a work which was the first great cytological publication in this hemisphere. This monograph, "The Maturation, Fecundation and Segmentation of Limax campestris, Binney," published in 1881, was 448 pages in length with 5 beautiful double lithographed plates made from Professor Mark's careful drawings of living, fixed, whole, and sectioned ova of

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Limax. It reviewed the entire field of cytology up to that time. In this work he advanced the idea that polar cells were abortive ova. The work was done with the care, neatness and accuracy which characterized all Dr. Mark's activities throughout his life. He would never tolerate the least sloppiness either of technique or expression.

In 1885 he was made Hersey Professor of Anatomy, and not long after that he was guiding his students on a variety of research problems. Those were the years when Parker, Davenport, Ward, Kofoid, Castle, Jennings and Mayer, to mention only a few, were getting their Ph.D.'s at Harvard under Mark. Throughout his long service as professor and director he seldom missed his weekly consultation period with each graduate student. He critically examined each week's progress, carefully examined the candidate's microscope slides, discussed the literature and gave valuable suggestions for the progress of the problem. When the work was completed he used the same care in going over the manuscript for clarity of expression, accuracy of punctuation and bibliographic data. Each plate had to be clear, neat and pleasing in its composition and the labeling done just so. Many of Dr. Mark's critics have chided him for his insistence on accuracy of detail and orderliness but the record of the papers published under his editorship at Harvard and the Bermuda Biological Station will show that they were never hurried, poorly prepared or poorly documented contributions. He seldom put his own name in the authorship of the hundreds of papers to which he gave so much of his time, energy and thought.

He was loyal to his students and followed their triumphs and reverses with a kindly human interest. They too showed their affection for their professor in an extraordinary manner on three occasions. In 1903 they contributed original papers to the "Mark Anniversary Volume," an expression in the celebration of 25 years of successful work in the advancement of biology. In 1922 they celebrated his seventy-fifth birthday

in a banquet at Harvard and presented the university with a portrait of Mark. Again in 1937 on his ninetieth birthday he was presented with a scrap book of letters of appreciation written by his former students.

We owe much to the memory of Edward Laurens Mark for directly or indirectly all of us in anatomy have benefited much by the high standards he set for research and publications in America.

S. I. Kornhauser